

MEDICAL MICROBIOLOGY AND PARASITOLOGY

Prep Manual for Undergraduates

Third Edition

BS Nagoba, MSc (MED), PhD (MED)
Assistant Dean (Research & Development) and
Professor of Microbiology
Maharashtra Institute of Medical Sciences and Research, Latur
Maharashtra, India

Asha Pichare, MD (MICRO)
Professor and Head, Department of Microbiology
Maharashtra Institute of Medical Sciences and Research, Latur
Maharashtra, India

ELSEVIER

ELSEVIER

RELX India Private Limited

Registered Office: 818, 8th Floor, Indraprakash Building, 21, Barakhamba Road, New Delhi 110001

Corporate Office: 14th Floor, Building No. 10B, DLF Cyber City, Phase II, Gurgaon-122002, Haryana, India

Medical Microbiology and Parasitology: Prep Manual for Undergraduates, 3rd Edition, BS Nagoba and Asha Pichare

Copyright © 2016, 2012, 2007 by RELX India Private Limited
All rights reserved.

ISBN: 978-81-312-4427-2

e-Book ISBN: 978-81-312-4428-9

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Details on how to seek permission, further information about the Publisher's permissions policies and our arrangements with organizations such as the Copyright Clearance Center and the Copyright Licensing Agency, can be found at our website: www.elsevier.com/permissions.

This book and the individual contributions contained in it are protected under copyright by the Publisher (other than as may be noted herein).

Notice

Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

With respect to any drug or pharmaceutical products identified, readers are advised to check the most current information provided (i) on procedures featured or (ii) by the manufacturer of each product to be administered, to verify the recommended dose or formula, the method and duration of administration, and contraindications. It is the responsibility of practitioners, relying on their own experience and knowledge of their patients, to make diagnoses, to determine dosages and the best treatment for each individual patient, and to take all appropriate safety precautions.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of product liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

Although all advertising material is expected to conform to ethical (medical) standards, inclusion in this publication does not constitute a guarantee or endorsement of the quality or value of such product or of the claims made of it by its manufacturer.

Please consult full prescribing information before issuing prescription for any product mentioned in this publication.

Sr Project Manager-Education Solutions: Shabina Nasim

Manager Content Strategy: Renu Rawat

Snr Content Development Specialist: Goldy Bhatnagar

Project Manager: Nayagi Athmanathan

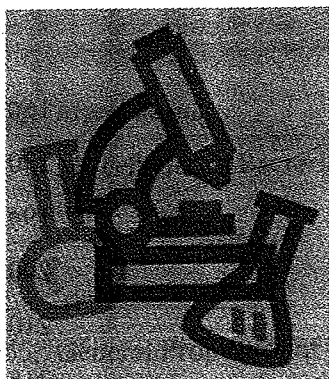
Manager Publishing Operations: Sunil Kumar

Snr Production Executive: Ravinder Sharma

Snr Graphic Designer: Milind Majgaonkar

Typeset by GW India

Printed in India by Rajkamal Electric Press, Kundli, Haryana



Contents

Preface to the Third Edition

vii

Preface to the First Edition

viii

Acknowledgements

ix

Unit I: General Microbiology

1. Introduction	3
2. History of Microbiology	6
3. Microscopy and Staining Techniques	11
4. Morphology of Bacteria	19
5. Physiology of Bacteria	29
6. Classification of Bacteria	36
7. Culture Media	40
8. Culture Methods	47
9. Identification of Bacteria	54
10. Sterilization and Disinfection	64
11. Hospital Waste Management	79
12. Bacterial Genetics	84
13. Infection	102
14. Normal Microbial Flora	110
15. Antimicrobial Susceptibility Testing	115

Unit II: Immunology

16. Immunity	123
17. Antigen (Ag)	131
18. Immunoglobulins (Igs)—Antibodies (Abs)	135
19. Antigen–Antibody Reactions	142
20. The Complement System	160

21. Structure and Functions of Immune System	166
22. The Immune Response	180
23. Immunodeficiency Diseases	194
24. Hypersensitivity (Allergy)	200
25. Autoimmunity	210
26. Transplantation and Tumour Immunity	215
27. Immunization, Immunizing Agents, Immunoprophylaxis and Immunotherapy	222

Unit III: Systemic Bacteriology

28. Laboratory Diagnosis of Bacterial Diseases	229
29. <i>Staphylococcus</i>	236
30. <i>Streptococcus</i>	244
31. <i>Pneumococcus</i>	252
32. <i>Neisseria</i>	257
33. <i>Corynebacterium</i>	265
34. <i>Bacillus</i>	272
35. <i>Clostridium</i>	278
36. Nonsporing Anaerobes	291
37. Enterobacteriaceae I: <i>Escherichia coli</i> , <i>Klebsiella</i> and <i>Proteus</i>	297
38. Enterobacteriaceae II: <i>Salmonella</i>	312
39. Enterobacteriaceae III: <i>Shigella</i>	324
40. <i>Pseudomonas</i> and <i>Burkholderia</i>	329
41. <i>Vibrio</i>	334
42. <i>Yersinia</i> , <i>Pasteurella</i> and <i>Francisella</i>	341
43. <i>Bordetella</i>	347
44. <i>Brucella</i>	352
45. <i>Haemophilus</i>	357
46. Mycobacteria I: <i>Mycobacterium Tuberculosis</i>	363
47. Mycobacteria II: Atypical Mycobacteria	374
48. Mycobacteria III: <i>Mycobacterium Leprae</i>	378
49. Spirochaetes	384
50. <i>Actinomyces</i> and <i>Nocardia</i>	396
51. Rickettsiaceae and Bartonellaceae	400
52. <i>Chlamydiae</i>	407

53. <i>Mycoplasma</i>	412
54. Miscellaneous Bacteria	416

Unit IV: Virology

55. General Properties of Viruses	421
56. Virus-Host Interactions (Virus Infection)	438
57. Laboratory Diagnosis of Viral Diseases	447
58. Bacteriophages	451
59. Poxviruses	456
60. Herpesviruses	460
61. Adenoviruses	472
62. Picornaviruses	475
63. Orthomyxoviruses	481
64. Paramyxoviruses	488
65. Arboviruses	496
66. Rhabdoviruses	502
67. Hepatitis Viruses	509
68. Oncogenic Viruses	520
69. Human Immunodeficiency Virus (HIV)	523
70. Miscellaneous Viruses	531

Unit V: Mycology

71. Introduction to Mycology	541
72. Superficial Mycoses	552
73. Deep Mycoses	561
74. Opportunistic Mycoses	583
75. Miscellaneous Mycoses	591

Unit VI: Parasitology

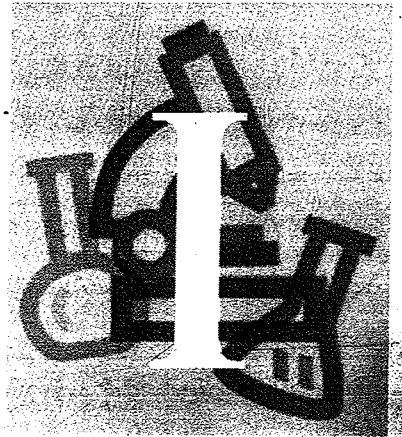
76. Introduction to Parasitology	597
77. Medically Important Amoebae	602
78. Medically Important Flagellates	610
79. Malarial Parasites	619

80. Other Medically Important Protozoan Parasites	626
81. Helminths: Medically Important Intestinal Cestodes	633
82. Medically Important Trematodes	641
83. Medically Important Intestinal Nematodes	649
84. Medically Important Tissue Nematodes	660

Unit VII: Clinical Microbiology

85. Septicaemia and Bacteraemia	669
86. Pyrexia of Unknown Origin (PUO)	673
87. Meningitis	678
88. Respiratory Tract Infections (RTI)	682
89. Diarrhoeal Diseases	689
90. Urinary Tract Infections (UTI)	695
91. Sexually Transmitted Diseases (STDs)	700
92. Hospital Acquired Infections	704
93. Zoonoses	710
94. Bacteriology of Water, Milk and Air	714
<i>Index</i>	725

UNIT I



General Microbiology

1

Chapter

Introduction

■ What is the difference between microorganisms and macroorganisms?

The earth on which we are living is an excellent place for the existence of different forms of life such as animals, human beings, plants, which are visible to naked eye and are called macroorganisms. In addition, there is existence of other forms of life, which are invisible to naked eye. These organisms are called microorganisms (micro = small, bios = life).

■ What does Microbiology deal with?

The science that deals with the study of microorganisms and their activities is called **Microbiology**. The microorganisms are studied with reference to their

- form and structure
- reproduction
- physiology and metabolism
- identification
- distribution in nature
- relationship with each other and with other living organisms, etc.

■ Group microorganisms on the basis of their morphology and functional properties.

- All microorganisms are not alike. Some of them are very small while others are relatively large in size
 - Some possess plant-like characters, while others have animal-like characters and few of them are altogether different
- Based on morphological and functional properties, they are grouped as
 - **Bacteria** are small, unicellular, microscopic organisms with primitive nucleus
 - **Fungi** are unicellular or multicellular microscopic organisms with well-developed nucleus. They possess plant-like characters but are devoid of chlorophyll and are not differentiated into roots, stem, leaves, etc.
 - **Algae** unicellular or multicellular microscopic organisms possessing plant-like characters. They possess chlorophyll but are not differentiate into roots, stem, leaves, flowers, etc. Most of them possess well-developed nucleus, except blue green algae
 - **Protozoa** are unicellular, nonphotosynthetic microscopic organisms possessing animal-like characters, i.e. they do not possess rigid cell wall. They have a well-developed nucleus
 - **Viruses** are very small, ultramicroscopic (seen under electron microscope), noncellular microorganisms capable of multiplying only inside the living cell. They are different from other living creatures

- To which taxonomic kingdom do microorganisms belong? Name the subdivisions of kingdom of microorganisms on the basis of cellular organization.

In early history, living organisms were classified into two kingdoms:

1. Plant kingdom and
2. Animal kingdom

After their discovery, microorganisms were initially placed in either plant or animal kingdom based on their characters. With the increase in the knowledge of microorganisms, it was found that many microorganisms possess both plant-like and animal-like characters or neither plant-like nor animal-like characters. Because of this problem, a new kingdom *Protista* was proposed for microorganisms by Ernst Haeckel (1866). All bacteria, fungi, algae and protozoa are grouped in the kingdom *Protista*. Viruses are not included because they do not have cellular organization.

The kingdom *Protista* is further divided into two groups based on structural (cellular organization) differences among the microorganisms, as

- Prokaryotes
- Eukaryotes

- Enumerate the differences between prokaryotes and eukaryotes.

The differences between *prokaryotes* and *eukaryotes* are presented in Table 1.1.

Table 1.1 Differentiating features of prokaryotes and eukaryotes

	Prokaryotes	Eukaryotes
1. Size	Less than 5 μ	Greater than 5 μ
2. Nucleus	Primitive type	Well developed
Nuclear membrane	Absent	Present
Nucleolus	Absent	Present
Deoxyribonucleoprotein	Absent	Present
Chromosome	Single (circular)	One or more (linear)
Mitotic division	Absent	Present
3. Cytoplasm		
Mitochondria	Absent	Present
Golgi bodies	Absent	Present
Endoplasmic reticulum	Absent	Present
Ribosome	70 S	80 S
Lysosome	Absent	Present
4. Chemical composition		
Sterols in plasma membrane	Absent	Present
Cell wall	Complex	Simple
Peptidoglycan	Present	Absent
Muramic acid	Present	Absent
5. Other		
Respiration	Part of plasma membrane (mesosomes)	Mitochondria
6. Examples	Bacteria, blue green algae	Algae, fungi, protozoa

- Discuss the beneficial and harmful effects of microorganisms on human, animal and plant life.

Microorganisms are beneficial as well as harmful to human beings, animals and plants. Although, the harmful effects of microorganisms are emphasized, beneficial effects are more than harmful effects.

Beneficial Effects

Many microorganisms are useful/beneficial in diverse ways, viz.:

- Many of them produce organic acids like citric acid, lactic acid, acetic acid, etc., which are useful to us in many different ways
- Some of them produce vitamins, amino acids, enzymes, etc. and are used for production of these substances, which are essential for us
- Certain microorganisms produce wines and alcohols
- Some microorganisms are able to fix atmospheric nitrogen into organic matter and thus help to increase the soil fertility
- Methanogenic bacteria produce methane gas, which is a biofuel
- Some microorganisms produce antibiotics, which are used for the treatment of infectious diseases caused by pathogenic microorganisms

Harmful Effects

Many microorganisms are harmful in different ways, viz.:

- Many microorganisms cause disease in man, animals and plants. Though considerable success has been achieved in the control of many microorganisms causing diseases, microorganisms still pose serious threats to human beings
- Many of them spoil our food
- Some of them spoil water—produce odour and make water unsafe for drinking
- Some microorganisms spoil books, leather, furniture, rubber, iron pipes, wall paints, etc.

- What does Medical Microbiology deal with?

It is the branch of Microbiology that deals with the study of disease-producing microorganisms in human beings. It also includes prevention and control of the disease.

- List the various branches of Medical Microbiology.

The various branches of Medical Microbiology are:

- General Microbiology
- Immunology
- Bacteriology
- Virology
- Mycology
- Parasitology
- Clinical Microbiology

2

Chapter

History of Microbiology

■ Discuss the evolution of the concept of microbes and their generation.

The concept that contagious disease was caused by invisible living things was known from ancient time but the definitive knowledge about microbes had to await till microscopes were developed.

- Varo and Columella in the 1st century BC postulated that invisible organisms, which they called *Animalia minuta*, caused diseases
- Fracastorius of Verona (1546) proposed *Contagium vivum* as the possible cause of infectious disease
- von Plenciz (1762) suggested that a separate agent caused each disease
- Aristotle proposed that animals and plants originated from spontaneous combination of elements and ethereal principles (theory of spontaneous generation—**abiogenesis**)
- Redi (1688) experimentally proved that life comes from life. However, Needham (1745) supported Aristotle's theory of spontaneous generation (abiogenesis) and confirmed experimentally spontaneous generation of microorganisms from the decomposing organic matter
- Spallanzani (1769) did not support the theory of abiogenesis and found that no such generation of microbes occurs spontaneously in broth subjected to high temperature
- Pasteur proved conclusively that all forms of life, even microbes, arose only from their like and *de novo*

■ Discuss the important contributions of Antony van Leeuwenhoek (1632–1723) in the field of microbiology.

- The credit for observation and description of microbes goes to Antony van Leeuwenhoek
- He was a Dutch draper whose hobby was to prepare lenses and to observe diverse materials through them
- He was able to **produce an instrument having a magnification of 40–300** through which he observed minute organisms in rainwater and other materials and designated them as **animalcules**
- He communicated his findings to the Royal Society of London in 1676. However, he did not realize the importance of these organisms
- He was first to observe *Giardia lamblia*—an intestinal flagellate in his own stool in 1681
- In 1678, Robert Hook developed a compound microscope and confirmed Leeuwenhoek's observations
- Though many people considered animalcules as the cause of contagious diseases, the idea was finally accepted and firmly established as a scientific fact almost after a century
- The present day science of Microbiology is greatly influenced by the contributions of Louis Pasteur and Robert Koch

■ List the important contributions of Louis Pasteur (1822–1895).

- **Louis Pasteur** was a French chemist. His discovery of fermentation built up his interest in further research into microbiology
- He is known as the **Father of Modern Microbiology**. His important contributions are as follows:
 1. In 1857, he established that fermentation was the result of microbial activity
 2. Introduced the techniques of sterilization, e.g., steam sterilizer, autoclave, hot air oven, flaming, pasteurization
 3. Established the **importance of cotton plugs** for protection of culture media from aerial contamination
 4. During his work on pébrine—a disease of silk industry, **protozoal aetiology** was discovered
 5. Introduced the use of **complex media**
 6. His accidental observation that culture of chicken cholera bacillus left on the bench for several weeks lost its pathogenic property but retained its ability to protect the birds against subsequent infection by chicken cholera bacilli led to the process of attenuation and development of **live vaccines** (1880)
 7. Prepared **anthrax vaccine** by attenuating anthrax bacilli at high temperature (42°–43°C) in 1881. He coined the term '**vaccine**' for such prophylactic preparations
 8. Developed a vaccine for Rabies in 1888

■ List the important contributions of Joseph Lister (1827–1912).

- Joseph Lister is known as the **Father of Antiseptic Surgery**
- He realized that microorganisms prevalent in the atmosphere might be responsible for postoperative wound infections
- He was interested in the prevention of postoperative sepsis
- Introduced **antiseptic techniques in surgery** (1867) using carbolic acid as a spray during operation or on the wound in postoperative stage. It was a revolution and an important milestone in surgical practice

■ What are the important contributions of Robert Koch (1843–1910)?

- Robert Koch is known as the **Father of Bacteriology**
- He was first to isolate anthrax bacilli in pure culture and to show **spores in anthrax bacilli** (1876)
- Introduced **staining techniques** and methods of obtaining **bacteria in pure culture** by using solid media
- Discovered **tubercle bacilli** (1882) and *cholera vibrio* (1883)
- Discovered **old tuberculin** in 1880–1901 but it was not universally accepted for the treatment of tuberculosis

✓ Koch's Postulates

Robert Koch postulated the criteria for proving that a microorganism isolated from a disease was indeed causally related to it.

According to these postulates, a microorganism can be accepted as the causative agent of the disease only if following conditions are satisfied.

- The microorganism should be *constantly associated* with the lesions of the disease
- It should be possible to *isolate the organism in pure culture* from the lesions of the disease

- Inoculation of such pure culture in suitable laboratory animals should produce a *similar disease in animals*
- It should be *possible to reisolate* the organism in pure culture from lesions produced in the experimental animals
- An additional criteria introduced subsequently requires that—*specific antibodies* to that organism should be demonstrable in the serum of patients

These postulates have proved to be useful in confirming doubtful claims made regarding the causative agents of infectious diseases.

Exceptions to Koch's Postulates

It is not always possible to satisfy all the postulates in every case. Some exceptions are:

- *Treponema pallidum* and *Mycobacterium leprae* are unable to grow on artificial media
- Many viruses and Rickettsiae are unable to grow on artificial media
- Who is known as the Father of Chemotherapy? List his important contributions.
 - **Paul Ehrlich** is known as the Father of Chemotherapy
 - He was first to launch search for **magic bullet**—a chemotherapeutic agent that destroys a pathogen without causing any harm to the infected host
 - In 1900s, he cured trypanosomiasis in rats and mice by using **trypan red** (dye) and **atoxyl** (organic arsenic compound)
 - In 1906, he discovered **Salvarsan**—an arsenic compound for the successful treatment of syphilis in early phase

- Who discovered Prontosil as an antibacterial agent?

In 1935, Domagk discovered Prontosil (sulphonamides) active against specific bacteria.

- Mention the contributions of the following in the discovery of antibiotics:
(a) Sir Alexander Fleming (1928), (b) Chain (1940), and (c) Waksman (1944).
- In 1928, **Sir Alexander Fleming** accidentally discovered antibacterial effect of *penicillin* produced by mold *Penicillium*
- Subsequently, *stable and safe preparations* of penicillin were developed by **Chain et al.** in 1940
- This discovery was the initiation of the antibiotic era and large numbers of other antibiotics were discovered in rapid succession using *fungi* as source of anti-microbial agents including streptomycin by **Waksman et al.** in 1944

- In a tabulated form list chronologically (from 1850–1896) names of scientists and their important discoveries.

Chronological listing of scientists and their important discoveries are presented in Table 2.1.

- Discuss with examples, the discovery of viruses.

By the end of 19th century many infectious diseases were proved to have bacterial aetiology. But there were large number of diseases for which no bacterial cause could be established. These included small pox, chickenpox, measles, common cold, etc. During the studies on rabies, Pasteur suspected that the causative agent could be too small to be seen under microscope.

- **Ivanovsky** proved the existence of such ultramicroscopic microorganisms in 1892 when he reproduced a mosaic disease in tobacco plant by applying the juice from diseased plants to healthy leaves from which all bacteria had been removed

Table 2.1 Scientists and their contributions

Year	Scientists	Discoveries
1850	Davaine and Pollender	Anthrax bacilli
1874	Hansen	Lepra bacilli
1879	Neisser	Gonococci
1881	Ogston	Staphylococci
1882	Robert Koch	Tubercle bacilli
1883	Robert Koch	Cholera bacilli
1884	Loeffler	Diphtheria bacillus
1885	Nicolaier	Tetanus bacilli
1886	Frankel	Pneumococci
1886	Escherich	<i>E. coli</i>
1887	Weichselbaum	Meningococci
1887	Bruce	<i>Brucella</i>
1892	Pfeiffer	Influenza bacilli
1892	Welch and Nuttall	<i>Clostridium welchii</i>
1894	Yersin and Kitasato	Plague bacilli
1896	Shiga	<i>Shigella</i>

Beijerinck (1898) confirmed these findings

In 1898, **Loeffler and Frosch** demonstrated that a filterable agent is responsible for foot and mouth disease

The first human disease proved to have a viral aetiology was yellow fever. Its nature and transmissibility through the bite of infected mosquitoes was established by **Walter Reed** (1902) in Cuba

Landsteiner and Propper (1909) demonstrated causative agent of poliomyelitis and were also successful in transmitting the virus to the monkeys

In 1934, **Ruska** developed electron microscope—which made morphological examination of even small viruses possible

Cultivation techniques for viruses were developed in 1930s—chick embryo technique by **Good Pasteur** and by 1940, tissue culture methods were developed

The possibility that infection with virus could lead to malignancy was first put forth by **Ellerman and Bang** (1908)

Towart (1915) and **Félix d'Herelle** (1917) independently discovered bacteriophages

Tabulate important discoveries in virology by **Smith** (1933), **Hyashi** (1934), **Row** (1953), **Montagnier** (1983), **Robert Gallo** (1984).

The important contributions of **Smith** (1933), **Hyashi** (1934), **Row** (1953), **Montagnier** (1983), **Robert Gallo** (1984) are given in Table 2.2.

Apart from contributions of Pasteur in the development of vaccines for immunization, mention other important historical landmarks in immunology.

In addition to the contributions of Pasteur, other important landmarks in immunology are:

Edward Jenner (1796) carried out the first successful immunization against smallpox by using cowpox virus. His observations that exposure of milkmaids to occupational cowpox infection makes them immune to small pox led to the techniques of vaccination

Nuttall (1888) observed that *defibrinated blood* had a bactericidal effect

Buchner (1889) noticed that *heating the sera* for one hour at 55°C destroyed this effect

Table 2.2. Important discoveries by the above-mentioned scientists in virology

Year	Scientist	Discovery
1933	Smith	Influenza virus
1934	Hyashi	Japanese encephalitis virus
1953	Row	Adenovirus
1983	Montagnier	Lymphadenopathy associated virus
1984	Robert Gallo	HIV

- **von Behring and Kitasato** (1890) described a *specific humoral factor*—antibody produced in guinea pig's serum by injecting a series of sublethal doses of tetanus toxin
- **Pfeiffer** (1893) demonstrated bactericidal effect *in vivo* by injecting live cholera vibrio into specifically immunized guinea pigs (*bacteriolysis in vivo*)
- **Bordet** (1895) established that bacteriolysis *in vivo* required two factors—the heat stable antibody and heat labile factor, which was called alexine by Buchner (1889), subsequently named as complement by Ehrlich
- **Metchnikoff** (1883) proposed that the *phagocytic response as the prime defense mechanism* against microorganisms invading blood and tissues and thus the concept of cellular immunity was established
- **Wright** described *opsonin* in serum in 1903
- The discipline of allergy and its importance in the pathogenesis of human disease came into existence after the experiment of **Portier and Richet** (1902) on dog that led to the discovery of *anaphylactic reaction*
- In 1955, **Jerne** proposed the '*natural selection*' theory of antibody synthesis and **Burnet** (1957) modified this into the *clonal selection theory*
- **Burnet** (1967), proposed the concept of *immunological surveillance*, which postulates that the primary function of the immune system is to preserve the integrity of the body, seeking and destroying all the foreign antigens including malignant cells arising by somatic mutation. Development of malignancy was visualized as a failure of immunological surveillance
- **Medawar et al.** in 1940s proved the *immunological basis of transplantation*.

3

Chapter

Microscopy and Staining Techniques

- What is a microscope and how is it important in the study of microbes?
 - A microscope is an optical instrument, consisting of a lens or combination of lenses for making enlarged or magnified images of minute objects
 - Microbes such as bacteria are too small to be seen by the unaided eye, therefore they must be magnified to be seen and studied. This is done by using microscopes. Microscopes of different types are required for their magnification
 - The limit of resolution with the unaided eye is about 200 μ . Bacteria are much smaller. Medically important bacteria generally measure 0.2–1.5 μ in diameter and 3–5 μ in length. Hence, they must be greatly magnified for observation and study of their structure and properties
- Give the unit(s) of measurement of microbes. Also give the conversion table for these.

The size of microbes is measured in unit of a micron (micrometer) or millimicron (nanometers).

CONVERSION TABLE

1 micron (μ) or micrometer (μm) = 1/1000 millimeter
 1 millimicron ($\text{m}\mu$) or nanometer (nm) = 1/1000 μm (micron) or one millionth of mm
 1 angstrom unit (\AA) = (1/10) of nm (nanometer)

- Based on the principle of magnification, name two types of microscopes.

The two types of microscopes based on the principle of magnification are:

1. Light or Optical Microscope

Microscopy in which magnification is obtained by a system of optical lenses. These include:

- Bright field microscope
- Dark field microscope
- Fluorescent microscope
- Phase contrast microscope

2. Electron Microscope

Microscopy in which magnification is obtained by a beam of electrons.

- State the working principle and uses of various types of light/optical microscopes, and the electron microscope.

The working principle and uses of microscopes are described below:

1. Bright Field Microscope (Compound Microscope)

This microscope contains a light source and compound lens system. The lens system consists of

Objective Lenses

These are of the following three types:

1. Low power (10X) used for examination of protozoa and other larger micro-organisms. It gives total magnification of 100X (10×10 of eyepiece)
2. High power (40X) used for examination of bacterial motility, protozoa, fungi, etc. It gives total magnification of 400X (40×10)
3. Oil immersion (100X) used for examination of stained smears of bacteria. It gives total magnification of 1000X (100×10). The use of oil (immersion oils) having same refractive index to fill the space between object and objective avoids refraction of rays of light and pass them directly into the objective. This gives resolution of 0.2μ approximately

Eye Piece (Ocular lens)

It is a fixed lens, usually 10X. It magnifies the real image of the object formed by the objective.

In addition to these important lenses, microscope also has

- Condenser—its lenses focus light from the illuminating source on the plane of the object
- Iris diaphragm—it is situated below the condenser to control the amount of light reaching the object and to regulate the path of light
- Mirror—to collect and reflect the light on object. It has two surfaces—concave and flat. Concave surface is used when light source is artificial and flat surface is used when light source is natural

2. Phase Contrast Microscope

- This microscope improves the contrast and makes evident the structure within the cells that differ in thickness or refractive index. Also, the differences in refractive index between bacterial cells and the surrounding medium make them clearly visible.
- When rays of light are passed through an object, they emerge in different phases depending on the difference in the refractive indices between the object and the surrounding medium and these phase differences are converted into differences in intensity of light producing light and dark contrast in the image.
- A special condenser with an annulus and a special phase objective with a phase plate are required for these differences. A light microscope can be converted into phase contrast microscope by using a special type of condenser and objective.

Uses

1. It is a method to observe unstained living organisms with good contrast and high resolution, e.g. *Vibrio cholerae* in stool specimen and cultures, *Entamoeba histolytica* in stools, *Trichomonas vaginalis* in specimens and cultures
2. Useful for study of structures and structural changes in larger microorganisms and tissue cells but not for small or slender objects like spirochaetes

3. Dark Field Microscope

- In this type of microscope, a special type of dark field condenser with a central circular stop is used, which illuminates the object with a cone of light without allowing light rays to enter directly the objective lens and only light rays reflected or scattered from the object enter the objective lens with the result that the object appears self-luminous against a dark background.
- An ordinary microscope can be converted into dark ground microscope by using (a) a special type of dark-field condenser with a central circular stop, and (b) a funnel stop for insertion in the 100X objective.

Uses

1. To demonstrate extremely slender organisms like spirochaetes
2. To demonstrate *Vibrio* and *Campylobacter* in specimens and cultures
3. To demonstrate microfilaria in blood—the sheath of pathogenic microfilaria can easily be seen

4. Fluorescent Microscope

- Fluorescence is the property of absorbing light rays of one wavelength and emitting (convert them into) light rays of another wavelength. This property is used in fluorescent microscope.
 - In this, the specimen is exposed to ultraviolet (UV) light of shorter wavelength that results in emission of longer wavelength of visible light and organisms stained with fluorescent dyes become self-luminous and are seen as bright objects against a dark background
 - Due to shorter wavelength of UV light, the resolving power can be proportionately extended

Uses

1. To visualize *Mycobacterium tuberculosis*, intracellular *Gonococci* and *Meningococci*
2. Detection of antigen in tissue or specimen
3. Detection of antibodies in serum

5. Electron Microscope

- In this microscope, instead of ordinary light, a beam of electrons is used.
- The wavelength of electron is approximately 0.005 nm, as compared to 500 nm of visible light.
- The resolving power of microscope is directly related to the wavelength. Thus, the resolving power of electron microscope is theoretically 100,000 times that of light microscope, but in practice it is about 0.1 nm.
- The electron microscope has the advantage of tremendous magnification and is used to study various fine structures of bacteria and viruses.

Uses

To visualize very small organisms such as viruses or structures of microbial cells, which are smaller than 0.2μ and cannot be observed under the light microscope.

■ **Mention the importance of staining in the study of microorganisms.**

Microorganisms are transparent or semitransparent with very little contrast between their cells and the surrounding medium. This makes it difficult to see them in an unstained state. When stained, they become opaque and the contrast between them and the surrounding medium is increased (as the surrounding medium remains transparent). As a result they become clearly visible.

■ **Name the techniques used for staining live and fixed (killed) organisms. Explain the types of staining techniques employed for killed organisms.**

Techniques for Staining Microorganisms

- **Vital staining**—The staining techniques in which organisms are stained in living state
- **Supravital staining**—The staining techniques in which organisms are killed

Supravital staining are of the following four types:

1. **Simple staining** (Monochrome staining): Watery solution of a simple basic dye is used, e.g. methylene blue, or basic fuchsin. They provide the colour contrast but impart same colour to all microbes in the smear
2. **Negative staining** (Background staining): A drop of India ink or nigrosine is mixed with culture of an organism or material containing organisms (e.g. sputum or CSF) and a thin film is prepared and observed under the microscope. In this, the background is stained and organisms appear as colourless objects against a dark background. This method is used for demonstration of capsule and spirochaetes
3. **Impregnation staining**: Organisms, which are very thin to be seen under light microscope, are rendered visible by increasing their thickness by impregnating silver on their surface. This method is used for demonstration of organisms like spirochaetes and bacterial flagella
4. **Differential staining**: The staining methods, which differentiate two types of organisms, are known as differential staining. They impart different colour to different bacteria or bacterial structures, e.g. Gram stain and acid fast stain

■ **Write an account of the microscopic methods used for unstained and stained preparations commonly used in the study of microorganisms.**

Unstained Preparations

1. Wet Mount

- It is an unstained preparation in which specimen can be observed directly, e.g. urine or in an emulsified suspension, e.g. stool

Procedure

- A drop of urine or some other specimens that does not require emulsification is taken on glass slide
- Cover slip is placed over it and the slide is observed under low power and high power or

A drop of saline is placed on a glass slide and specimens like stool, which require emulsification, are placed in it. Here saline acts as an emulsifying agent.

- Cover slip is placed on it and the slide is observed under low and high power

Uses

1. To assess and enumerate inflammatory cells
2. To examine urine deposits
3. To observe parasites such as *Trichomonas*, amoeba, *Giardia*, etc.

2. Hanging Drop Preparation**Procedure**

- * A drop of liquid culture or specimen such as stool is placed on cover slip and cover slip is inverted over a cavity slide so that drop remains hanging
- * This preparation is then observed under low power to adjust edge of the drop and under high power to study the motility of organisms

Uses

1. To study motility of *V. cholerae* in stool sample
2. To study motility of organisms in fluid culture

Stained Preparations**1. Gram Stain**

It is the most commonly used staining method devised by the histologist Christian Gram (1884) to stain bacteria in tissue.

- * It is a differential staining method, which differentiates organisms into Gram-positive and Gram-negative according to their Gram reaction

Procedure

- * Prepare smear from clinical specimen, culture smear of colony or broth culture on a clean glass slide, allow it to air dry and fix it by flaming.
- * **Primary staining:** Cover smear with crystal violet, methyl violet or gentian violet—allow it to act for 30–60 seconds
- * **Application of mordant:** Pour off the primary stain and apply Gram's iodine for 1–2 minutes and wash with water
- * **Decolourization:** Decolourize the smear with an organic solvent—absolute alcohol, acetone or aniline for 10–30 seconds or until the colour oozes from slide
- * Wash with water
- * **Counter staining:** Apply—safranin, carbol fuchsin or neutral red for 15–30 seconds
- * Wash with water and blot dry
- * Observe the slide under 100X after putting a drop of cedar wood oil

Observations

Gram stain divides bacteria in two broad groups:

1. **Gram-positive bacteria:** These bacteria resist decolourization and retain the colour of the primary stain and appear violet
Examples: Pneumococci, Streptococci and Staphylococci are Gram-positive cocci (Fig. 3.1)
Clostridia, Corynebacteria and *Bacillus* spp. are Gram-positive bacilli (Fig. 3.2)
2. **Gram-negative bacteria:** These bacteria are decolourized by alcohol and therefore, take counter stain and appear red
Examples: Gonococci and Meningococci are Gram-negative cocci (Fig. 3.3)
E. coli, *Salmonella*, *Shigella*, *V. cholerae*, etc. are Gram-negative bacilli (Fig. 3.4)
Background/pus cells and their nuclei appear red in colour



Fig. 3.1 Gram-positive cocci. (Source: *Laboratory Testing for Ambulatory Settings: A Guide for Health Care Professionals*, Fig. 7-9, pp. 219-254, Saunders, 2006.)

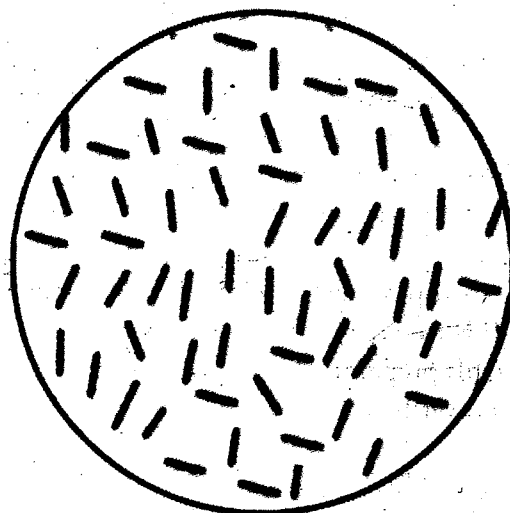


Fig. 3.2 Gram-positive bacilli.

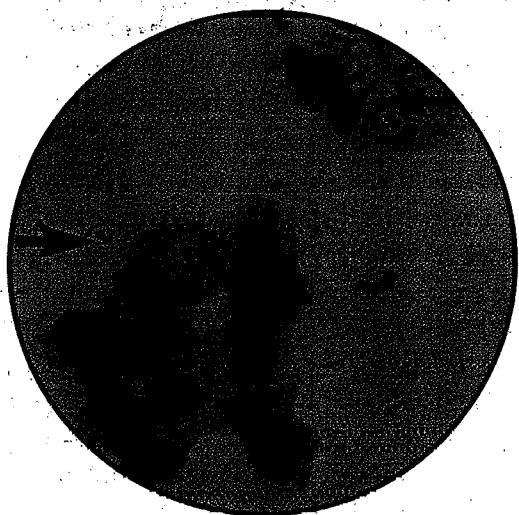


Fig. 3.3 Gram-negative cocci. (Source: *Laboratory Testing for Ambulatory Settings: A Guide for Health Care Professionals*, Fig. 8-12, pp. 236-273, Saunders, 2011.)

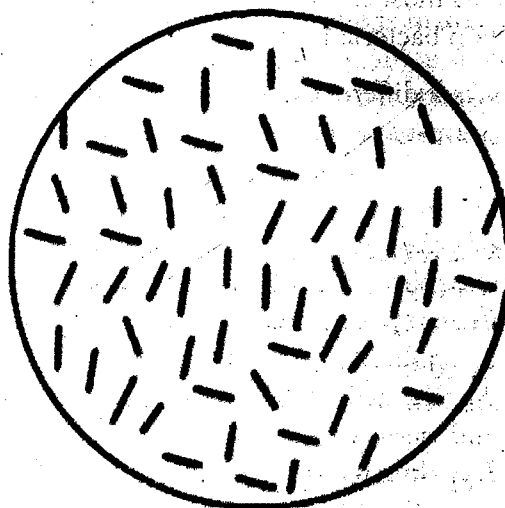


Fig. 3.4 Gram-negative bacilli.

The Gram reaction is not hard and fast distinguishing feature. The Gram-positive may appear Gram-negative because of

- Prolonged decolourization
- Damage to cell wall
- Death of bacteria
- The Gram-negative may appear Gram-positive because of inadequate decolourization

Mechanism of Gram reaction

Different theories that have been proposed to explain the mechanism are as follows:

- **Acidic protoplasm theory:** According to this theory, as compared to Gram-negative bacteria, Gram-positive bacteria have more acidic protoplasm and the primary stain used is

basic in nature. Hence, Gram-positive bacteria retain the primary stain more strongly than Gram-negative bacteria. Also the iodine makes the cytoplasm more acidic and acts as a mordant, increasing the attraction of the primary stain to the cell cytoplasm. Thus, it helps to fix the stain in bacterial cell

Lipid content theory: According to this theory, the lipid content of cell wall is more in Gram-negative bacteria and less in Gram-positive bacteria. During Gram reaction there is formation of primary stain-iodine complex in both Gram-positive and Gram-negative bacteria, which gives violet colour. However, when alcohol is applied, the lipid in the cell wall of Gram-negative bacteria gets dissolved in alcohol, leading to an increase in the pore size through which the dye-iodine complex diffuses out during the process of decolourization. On the other hand, the dye-iodine complex gets trapped within the Gram-positive cells because of fewer lipids and they thus retain the violet colour

Cell wall permeability: The cell wall of Gram-positive bacteria contain more mucopeptide because of which it is thicker and stronger, hence dye-iodine complex does not come out of the Gram-positive cell. However, in Gram-negative bacteria mucopeptide is less and therefore their cell wall is relatively less strong and thin, hence dye-iodine complex diffuses out of cell freely and they take up the colour of the counter stain

Magnesium ribonucleate theory: According to this theory, the magnesium ribonucleate is present in Gram-positive bacteria and there is formation of magnesium ribonucleate-dye-iodine complex, which is insoluble in alcohol. This component is, however, absent in Gram-negative bacteria and hence there is formation of dye-iodine complex only, which is soluble in alcohol. This makes the bacteria colourless

✓ 2. Acid Fast Stain (Ziehl-Neelsen Stain)

The organisms, which are not easily stained by ordinary staining methods, but once stained resist decolourization by acids are known as acid-fast organisms and method used for staining of these organisms is known as acid-fast stain

The method was devised by Ehrlich (1883) and modified by Ziehl-Neelsen (1885), hence it is known as Ziehl-Neelsen staining method

It is a differential staining method, which differentiates acid-fast organisms (mycobacteria) from other organisms (nonacid-fast)

Procedure

Prepare smear on a clean glass slide, allow it to air dry and fix it by flaming

Primary staining: Cover the smear with filtered carbol fuchsin and heat until steam rises. Do not boil; allow it to act for 5-10 minutes with intermittent heating

The stain must not be allowed to evaporate and dry on the slide. If necessary, add more stain to the slide and reheat. Heating is necessary for penetration of stain into the cell wall

Wash with water

Decolourization: Cover the slide with 20% sulphuric acid (H_2SO_4). The red colour of the smear changes to yellowish brown

Wash with water and observe the colour of the smear, if it is red, repeat decolourization till it becomes yellowish brown

Wash with water

Apply 90% alcohol for 2 minutes for decolourization (This step is optional and may be omitted, if specimen is not urine)

Wash with water

Counter staining: Counter stain with methylene blue or malachite green for 1-2 minutes

Wash, blot dry and observe under 100X after putting a drop of cedar wood oil

Observations

- * Acid-fast bacilli appear bright red (Fig. 3.5)
- * Pus cells, epithelial cells and other organisms appear blue or green

Mechanism of acid-fast stain

- * Acid-fast organisms are not easily stained because they are coated with lipids, fatty acids and higher alcohols in their cell wall
- * Mycolic acid (waxy substance) present in the cell wall does not allow the stain to penetrate easily inside these organisms
- * In Ziehl-Neelsen (Z-N) staining, carbol fuchsin (basic fuchsin + phenol), a phenolic solution of basic fuchsin, is applied with heat. Heat and phenol facilitate penetration of the dye
- * Subsequently, when decolourized by acid, the dye does not come out because it is soluble in phenol and phenol is more soluble in lipid substances (mycolic acid) hence there is no decolourization and they retain the colour of the basic fuchsin (primary stain)

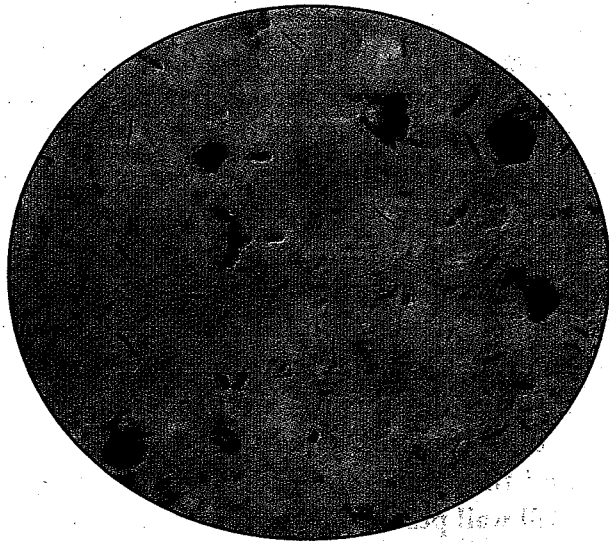


Fig. 3.5 Acid – fast bacilli.

4

Chapter

Morphology of Bacteria

■ What are bacteria?

Bacteria are unicellular prokaryotic microorganisms without chlorophyll. They do not show true branching except in higher bacteria (Actinomycetes).

■ Classify bacteria on the basis of their shape.

Depending on their shape, bacteria are classified into the following types (Fig. 4.1):

- Cocci (from Greek word *Kokkos*, meaning berry)—oval or spherical in shape
- Bacilli (from Latin word *bacillum*, meaning stick)—rod shaped
- Vibrios—comma shaped, curved rods
- Spirilla—Rigid spiral forms
- Spirochaetes (from Greek word *speira*, meaning coil and *chaite* meaning hair)—flexuous spiral forms
- Actinomycetes (from Greek word *aktis*, meaning ray and *mykes* meaning fungus)—branching filamentous bacteria resembling a radiating sun—rays-like appearance in tissue lesions
- Mycoplasma—cell wall deficient bacteria and hence do not possess stable shape

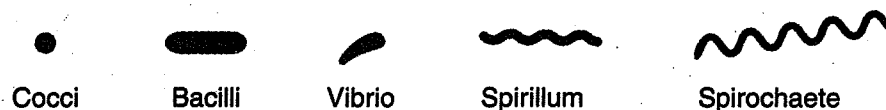


Fig. 4.1 Shapes of bacteria.

■ Classify bacteria based on characteristic arrangement of their cells.

Bacteria show characteristic arrangements (Fig. 4.2). Cocci may be arranged in

- Pairs, e.g. Diplococci
- Chains, e.g. Streptococci
- Groups of four, e.g. Tetrads (*Gaffkya*)
- Groups of eight, e.g. *Sarcina*
- Grape like clusters, e.g. Staphylococci
- Some bacilli also may be arranged in
- Chains, e.g. Streptobacilli, *Bacillus anthracis*
- Pairs, such as diplobacilli, e.g. *Klebsiella pneumoniae*
- Cuneiform or Chinese letter pattern, e.g. Corynebacteria

■ With suitable illustrations describe in detail a bacterial cell.

A typical bacterial cell (Fig. 4.3) shows the following structures:

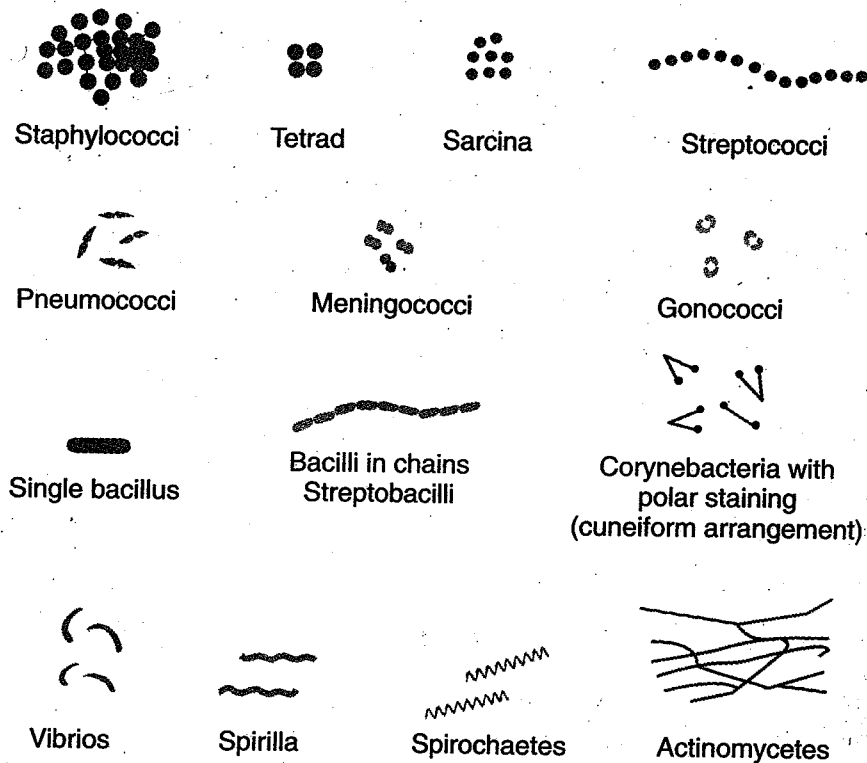


Fig. 4.2 Characteristic arrangement of bacterial cells.

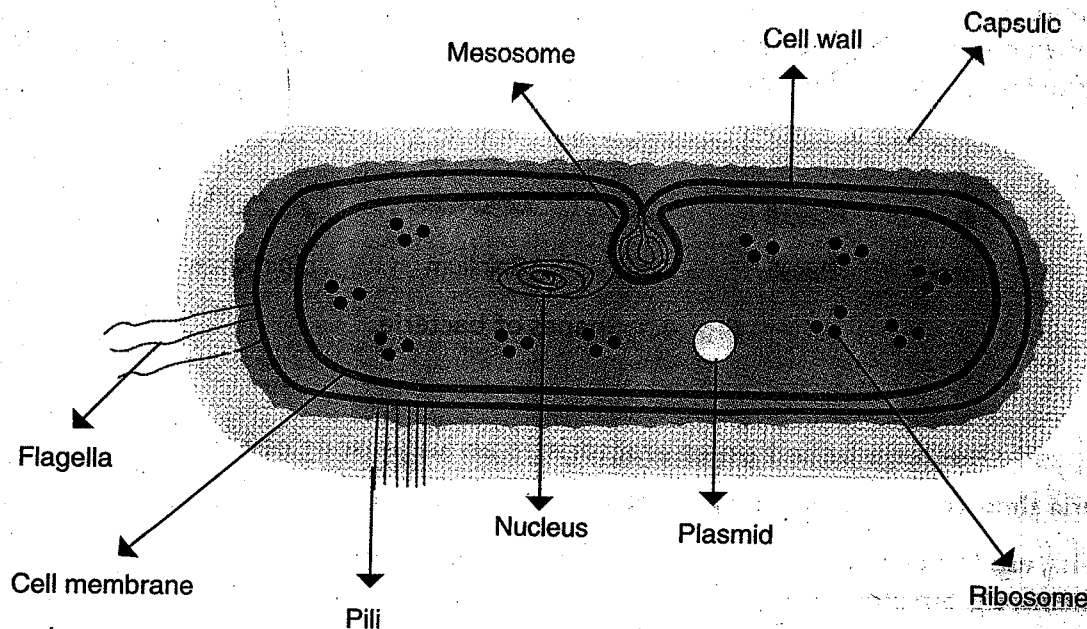


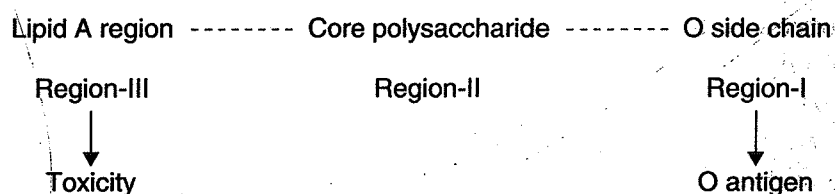
Fig. 4.3 Bacterial cell.

1. The Cell Wall

- It is a tough and rigid structure surrounding the bacterium like a shell
- It is responsible for the form of bacterial cell and plays a fundamental role in life activities of bacteria
- It is 10–25 nm in thickness and accounts for 20–30% of the dry weight
- Cell wall is absent in mycoplasma group of organisms

Chemical Nature

- Chemical composition of cell wall varies in Gram-positive and Gram-negative bacteria
- In both Gram-positive and Gram-negative bacteria, the component primarily responsible for mechanical strength is mucopeptide (peptidoglycan)
- The peptidoglycan is composed of alternating units of N-acetyl muramic acid and N-acetyl glucosamine linked together by beta—1—4 linkage and a set of tetrapeptide side chains attached to N-acetyl muramic acid. These monomeric units are cross-linked to each other by peptide cross bridges (Fig. 4.4)
- In Gram-positive bacteria, cell wall is 16–18 nm thick and monolayered. Peptidoglycan is the major component (50–80%), which is associated with polysaccharides and a special class of polymers called teichoic acids—glycerol teichoic acid or ribitol teichoic acid. The teichoic acid constitutes a major surface antigen in Gram-positive bacteria
- In Gram-negative bacteria, cell wall is comparatively thinner and a multilayered structure. It contains an inner layer of peptidoglycan (1–10%) about 2–3 nm and an outer layer (8–10 nm) known as outer membrane linked together by lipoprotein molecules. The outer membrane is composed of protein, phospholipids and lipopolysaccharide (LPS)—the proteins are embedded in a phospholipid bilayer, which is attached to LPS. These proteins are called outer membrane proteins, which form special channels called porins that permit passive diffusion of small molecules like sugars, amino acids, certain ions, etc. and serve as specific receptors for bacteriophages. The wall of some Gram-negative bacteria may possess a third layer of protein. LPS in Gram-negative bacteria consists of three regions:



Functions of Cell Wall

It plays fundamental role in life activities of bacteria:

- Gives rigidity and shape to cell
- Provides mechanical support to cell membrane

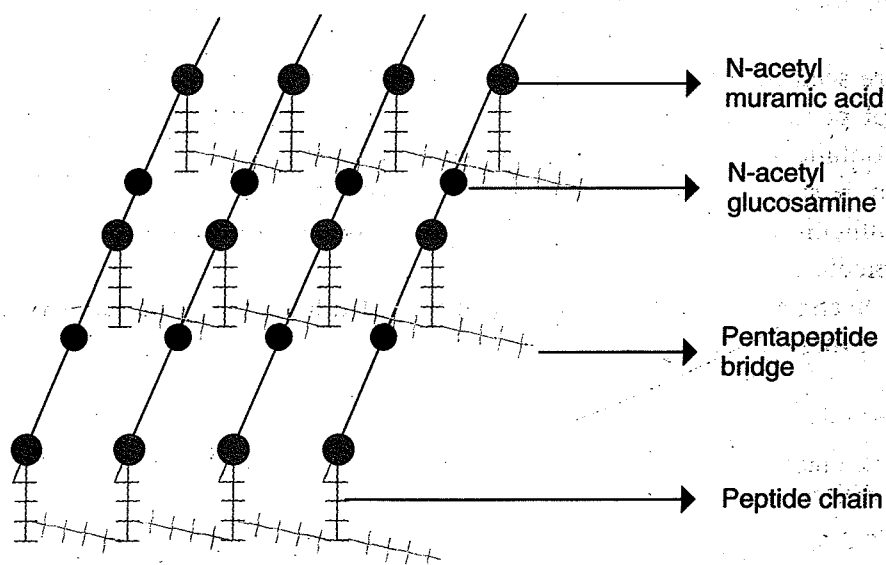


Fig. 4.4 Structure of peptidoglycan.

- Plays role in virulence and immunity
- Helps to maintain osmotic pressure and protects cell against osmotic damage
- LPS of Gram-negative bacteria have endotoxic activity and 'O' antigen specificity.
- Provides site for the phage absorption
- Takes part in cell division

Demonstration of Cell Wall

The presence of cell wall can be demonstrated by the following ways:

- Plasmolysis—when a bacterial cell is placed in hypertonic solution, the cytoplasm shrinks by losing water by osmosis, while cell wall retains original shape and size
- Microdissection
- Differential staining method
- Electron microscopy

Inhibition of Cell Wall Synthesis

Synthesis of cell wall may be inhibited by antibiotics, bacteriophages and lysozyme. Lysozyme is an enzyme normally present in many tissue fluids, which splits the beta 1-4 linkage of peptidoglycan in the cell wall.

Bacteria with defective cell wall obtained by the action of lysozyme are:

Protoplasts and spheroplasts

1. When lysozyme acts on Gram-positive bacteria in a hypertonic solution, protoplast containing only cytoplasmic membrane is formed
2. When lysozyme acts on Gram-negative bacteria, a spheroplast containing cytoplasmic membrane and some cell wall material is formed
3. Both protoplasts and spheroplasts obtained by the action of lysozyme are spherical in shape regardless of the original shape of the bacterium and require hypertonic conditions for their maintenance
4. These cell wall-deficient forms of bacteria—protoplasts and spheroplasts—may probably have a role in the persistence of certain chronic infections such as pyelonephritis

L-forms

- These are abnormal forms of bacteria first observed at Lister Institute, London hence named as L-forms
- L-forms are seen in many species of bacteria that develop either spontaneously or in the presence of penicillin or other agents that interfere with cell wall synthesis. Sometimes, formed spontaneously in patients treated with penicillin
- They are more stable than protoplasts and spheroplasts
- They are difficult to cultivate and require right osmotic strength for growth in agar containing solid medium
- They have been isolated from chronic urinary tract infection and suppurative infection but their role in these infections is not exactly known

2. Cytoplasmic (Cell) Membrane

- It is a thin, elastic, 5-10 nm, semipermeable layer lining the inner surface of the cell wall.
- It is a typical unit membrane structure composed of phospholipids and proteins, in which peptide molecules are embedded in phospholipid bilayer
- It also contains small amounts of carbohydrates but no sterols (except in *Mycoplasma*) and carrier molecules (enzyme permease, oxidase, polymerase, etc.)

Functions of Cell Membrane: Principal Osmotic Barrier

- Acts as a semipermeable membrane and controls the inflow and outflow of metabolites, i.e. it selectively allows passage of nutrients inside and waste products outside the cell
- With the help of enzyme permease, it participates in active transport of selective nutrients
- As it contains the cytochromes and other enzymes necessary for respiration, it acts as the center for respiratory activity to generate energy (ATP) by electron transport and oxidative phosphorylation
- Participates in the synthesis of cell wall components

3. Mesosomes

- Mesosomes are complex infoldings of the cell membrane
- Composed of an invaginated cell membrane with many vesicles or tubules filling the invagination
- They are chemically similar to cytoplasmic membrane with few other proteins
- They are more prominent in Gram-positive bacteria, also found in Gram-negative bacteria
- Mesosomes are of two types—septal and lateral
- Septal mesosome is attached to bacterial chromosome and is involved in DNA segregation and in the formation of cross walls during binary fission

Functions of Mesosomes

- Mesosomes are center for respiratory activity. They possess respiratory enzymes such as ATPase, dehydrogenase, cytochrome, etc. As mitochondria are absent in bacteria, respiratory activity is carried out by mesosomes
- They take part in cell division

4. Cytoplasm

- The bacterial cytoplasm is a colloidal system containing a variety of organic and inorganic solutes in a viscous watery solution
- It contains ribosomes, mesosomes, inclusion granules and vacuoles
- Endoplasmic reticulum and mitochondria are absent
- It stains uniformly in young cultures

a. Ribosomes

- The tiny granules, scattered in the cytoplasm are called ribosomes
- These are globular structures composed of RNA and proteins
- Made up of two subunits:
 - Larger subunit (50 S)
 - Smaller subunit (30 S)
- 10–15 nm in size with sedimentation coefficient of 70 S
- Function—site for protein synthesis

b. Inclusion granules

- These are nonliving bodies deposited in cytoplasm
- These are not permanent and essential structures usually deposited when large amount of nutrients are present and disappear under the conditions of starvation
- Examples: Lipid granules, volutin granules, sulphur granules
- These are source of stored energy and present in some species of bacteria

5. Nucleus

- The nucleus in bacteria is not well developed and is without nuclear membrane and nucleolus
- It contains a single, circular molecule of double stranded deoxyribonucleic acid (DNA) free from basic proteins and about 1 mm in length

Functions

It controls

- Growth and metabolism of cell
- Multiplication of cell
- Hereditary transmission of characters

In addition to nuclear DNA, bacteria may possess extrachromosomal genetic material consisting of DNA known as *plasmids* or *episomes*, which are not essential for life of the cell. They may be responsible for properties like drug resistance, toxigenicity, etc.

6. Capsule and Slime Layer

- Many bacteria synthesize organic polymers which are deposited outside the cell wall as a loose, gelatinous, amorphous viscid layer called capsule or slime layer
- When it is loose and irregularly arranged, it is called slime layer
- When it is organized into a sharply defined structure, it is known as capsule (Fig. 4.5)

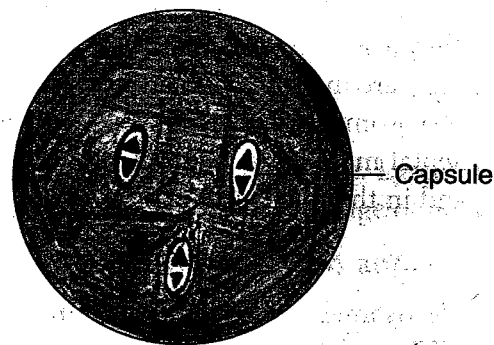


Fig. 4.5 Bacterial capsule.

Types

- **Macrocapsule:** Capsule that has a width of more than $0.2\ \mu$ and can be demonstrated by light microscope, is known as macrocapsule
- **Microcapsule:** Capsule that has a width of less than $0.2\ \mu$ and cannot be demonstrated by light microscope, is known as microcapsule

Chemical Nature

- Water is the main component: 98–99%
- Solids: 1–2%—generally carbohydrates, polysaccharides, but may be noncarbohydrate in nature
- Bacteria form capsule containing either homopolysaccharides (only one type of sugar) or heteropolysaccharides (more than one type of sugar)
- A few bacteria form capsule containing noncarbohydrate residues, e.g.
 - *Yersinia pestis*—a causative agent of plague forms capsule containing proteins
 - *Bacillus anthracis*—a causative agent of anthrax forms capsule containing D-glutamic acid (polypeptide)

Functions

- Enhances bacterial virulence by inhibiting phagocytosis
- Acts as protective covering against antibacterial action of substances such as lysozyme, colicins, bacteriophages, antibodies, etc.
- Acts as an antigen—helps in identification and typing of bacteria

Demonstration

Capsule can be demonstrated by

- Negative staining—using India ink or nigrosine
- Quellung reaction (capsule swelling reaction)

7. Flagella

- These are the thread-like structures arising from cytoplasm and extending out through cell wall
- They are contractile, extremely thin elongations about 5–20 μ in length and 0.01–0.02 μ in diameter
- All motile bacteria, except spirochaetes possess one or more flagella

Chemical Nature

Chemically, they are composed of a protein, known as flagellin (molecular weight—30,000–50,000).

Structure of Flagella

It consists of three distinct regions:

1. **Basal structure (basal body)**
 - a. Circular structure embedded in the cell envelope consists a central rod, which bears four rings—L, P and S, M
 - b. All four rings are present in Gram-negative bacteria and only S and M rings are present in Gram-positive bacteria indicating that only S and M are required for motility. L and P are required for support
 - c. In Gram-negative bacteria
 - L is situated in LPS
 - P is situated in inner peptidoglycan
 - S is situated just above the cell membrane
 - M is embedded in the cell membrane
2. **Hook:** Short curved structure, connecting the filament and basal body
 - It is broader than the filament, protein in nature different from flagellin and embedded in cell envelope
3. **Filament:** External to the cell and connected to hook at the cell surface
 - It is made up of flagellin (Fig. 4.6)

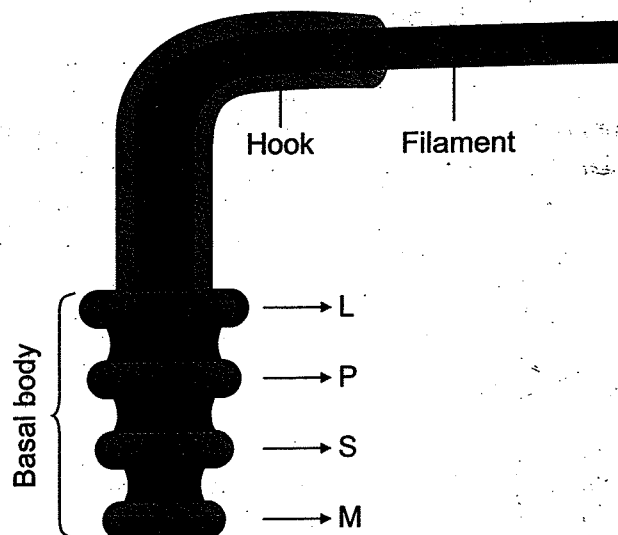


Fig. 4.6 Structure of bacterial flagellum.

Arrangement/types

- **Atrichate:** No flagella—nonmotile, e.g. *Klebsiella* spp.



- **Atrichate:** No flagella—motile, e.g. spirochaetes



- **Monotrichate:** Single flagellum at one end, e.g. *V. cholerae*



- **Amphitrichate:** Single flagellum at both the ends, e.g. *Alcaligenes faecalis*



- **Lophotrichate:** Tuft of flagella at one or both ends, e.g. *Spirilla* possessing flagella at both ends



- **Peritrichate:** Flagella all-round the cell, e.g. *E. coli*, *Salmonella typhi*

**Functions**

Flagella are locomotory organs responsible for motility (movement) which help the bacteria in following ways:

- To spread through the body fluids and tissues
- Active motility is necessary for uptake of nutrients
- In chemotaxis (movement towards attractant, e.g. food material), aerotaxis (movement towards oxygen) and phototaxis (movement towards light)
- Constitutes the flagellar (H) antigen

Demonstration

The following methods are used for demonstrating flagella:

- Electron microscope
- Special staining method—by increasing thickness (impregnation staining)
- Dark ground illumination

Presence of flagella can also be demonstrated by demonstrating motility by

- Hanging drop preparation
- Spreading growth on semisolid agar
- By using Craigie's or U tube technique

8. Pili or Fimbriae

- These are hair-like structures arising from cytoplasm in some Gram-negative bacteria and are different from flagella
- They are shorter in length (0.1–1 μ) and breadth (10 nm) as compared to flagella but are more in number than flagella
- Chemically they are protein—pilin, with molecular weight of 16,000
- Special type of pili—sex pili are present in male bacteria, which are longer (18–20 μ) than regular pili but are less in number (1–5 per cell while regular pili—several hundred per cell)

Functions

- Act as organ of adhesion—virulence factor
- Sex pili—transfer of genetic material from male to female by forming conjugation tube
- Help to form pellicle in liquid media
- Participate in haemagglutination reaction

Demonstration

Pili can be demonstrated by the following methods:

- Under an electron microscope
- Haemagglutination: Pili cause agglutination of red blood cells of guinea pigs, fowls, horses, pigs, sheep, humans, etc. Haemagglutination test is used for demonstration of pili in *E. coli*, *Klebsiella* and other bacteria

9. Bacterial Spores (Endospores)

- Spores are highly resistant resting phase of bacteria formed during abnormal environmental conditions
- They are resistant to heat, chemicals and other abnormal environmental conditions such as cold and desiccation
- Known as endospore—as spore is formed inside the bacterial cell
- Each bacterium forms one spore which on germination forms a single vegetative cell, hence it is not a method of reproduction
- Sporulating bacteria, e.g.
 - Aerobic, e.g. *Bacillus* spp.
 - Anaerobic, e.g. *Clostridium* spp.

Sporulation

- Sporulation occurs when nutritional conditions become unfavourable, i.e. during stationary phase of growth
- Sporulation begins with appearance of clear area in the protoplasm of the cell that becomes gradually opaque with condensation of nuclear material to form forespore. The cell membrane grows to form spore wall around it, which gets surrounded by cortex and multilayered tough spore coat. Exosporium may be formed in some spores

Properties of Endospores

- **Core:** It is a spore protoplast—contains nucleus, some unique enzymes, e.g. *dipicolinic acid* synthetase synthesizing *dipicolinic acid* (5–15%), which makes spore resistant to heat and other components
- **Spore wall:** It is the innermost layer surrounding the inner spore membrane—contains peptidoglycan—becomes future cell wall

- **Cortex:** It is the thickest layer surrounding the spore wall—contains unusual type of peptidoglycan with fewer cross-links, extremely sensitive to lysozyme
- **Coat:** Composed of keratin-like protein with many disulphide bonds. It is impermeable, which makes the spore resistant to antibacterial chemical agents
- **Exosporium:** Lipoprotein membrane containing some carbohydrates (Fig. 4.7)

Shape and Position of Spores

- Spore may be oval or spherical in shape
- Spore may be central, subterminal or terminal
- Spore may be bulging (diameter more than the bacterial cell) or nonbulging (diameter same as bacterial cell) (Fig. 4.8)

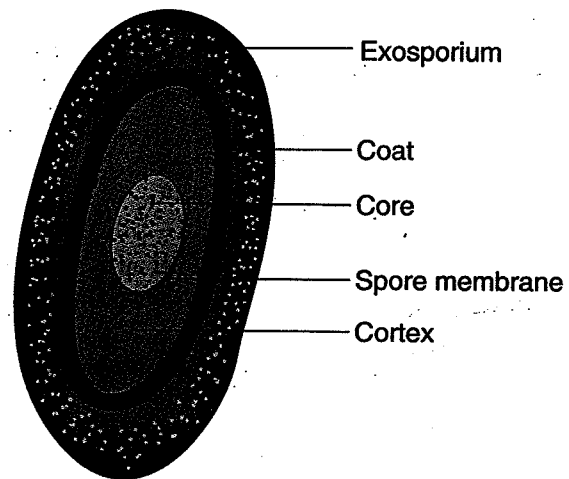


Fig. 4.7 Properties of endospores.

Germination of Spores

Germination occurs when conditions become favourable forming one vegetative cell. Three stages involved in germination are:

- **Activation:** It occurs in nutritionally rich medium. Damage to spore coat is produced by heat, abrasion, acidity, etc.
- **Initiation:** During this stage a cortex peptidoglycan and a variety of other components are degraded, water is taken up and calcium dipicolinic acid is released
- **Outgrowth:** A new vegetative cell with spore protoplast emerges out. A period of active synthesis occurs that terminates in cell division

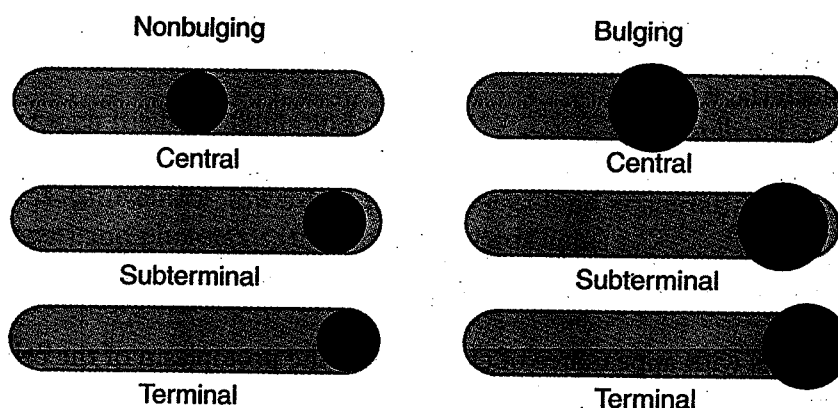


Fig. 4.8 Shape and position of spores.

5

Chapter

Physiology of Bacteria

- **Define the term Nutrition. Describe the two categories of nutrients required by bacteria for their growth and development.**

Nutrition is the process by which chemical substances, called nutrients, are obtained from surrounding environment and used for metabolic activity and growth of cell.

Bacteria require two categories of essential nutrients. These are:

1. **Macronutrients:** These are required in relatively large quantities and play important role in cell structure and metabolism
2. **Micronutrients:** These are required in small quantities for functioning of certain enzyme systems

- **What are the growth requirements of medically important bacteria? What is the meaning of term fastidious bacteria?**

Most of medically important bacteria grow on nutritionally simple media with only one source of organic material. However, some other bacteria are more exacting and require more complex media containing certain organic compounds known as fastidious bacteria.

- **Describe in detail optimum nutritional requirements for growth of bacteria.**

For optimum growth, bacteria require

1. Water
2. Source of carbon and nitrogen
3. Inorganic salts
4. Growth factors in some cases
5. Source of energy

Water

- It is the most important requirement because it is the principal constituent of a bacterial cell. It constitutes about 80% of the total weight
- It is vehicle for the entry of all nutrients into the cells and for the elimination of all waste products
- It participates in metabolic reaction
- It forms an integral part of the protoplasm

Carbon, Nitrogen and Energy Source

Bacteria are classified into four groups based on the carbon and nitrogen sources they utilize.

- **Autotrophs**—this group of bacteria is able to utilize atmospheric carbon dioxide and nitrogen to synthesize essential metabolites and because of this they are able to survive

independently in soil and water. They are concerned with soil fertility and are medically less important, e.g. nitrogen fixing bacteria in soil

- **Heterotrophs**—these are bacteria which require organic compounds as their source of carbon and energy—nutritional requirement varies widely and may require single organic compound or many organic compounds such as carbohydrates, amino acids, nucleotides, lipids and coenzymes. They are medically important, e.g. all disease causing bacteria
- **Phototrophs**—derive energy from the sunlight
- **Chemotrophs**—derive energy from the chemicals, e.g. *Nitrobacter*, *Nitrosomonas*

Inorganic Salts

These are required for osmotic regulation and to provide trace elements essential for certain enzyme systems. Particularly

- The anions—phosphate and sulphate
- The cations—sodium, potassium, magnesium, iron, manganese and calcium

Growth Factors

- Many pathogenic species require certain key substances for their growth known as growth factors or bacterial vitamins
- These include vitamins, purines, pyrimidines, amino acids, etc.

■ Discuss the environmental factors that affect growth of bacteria.

Environmental factors affecting bacterial growth are:

1. Moisture and Desiccation

Moisture is an absolute requirement for growth. The capacity to survive in dry environment varies from organism to organism. Some bacteria like Gonococci and *T. pallidum* die quickly in dry conditions, whereas *Staphylococcus aureus* and tubercle bacilli can survive drying for weeks and months.

2. Gaseous Requirements

Bacteria require oxygen for their growth. Based on oxygen requirements bacteria can be classified into four types, viz.

- **Obligate or strict aerobe**—grow in presence of oxygen
- **Microaerophilic**—require low oxygen concentrations
- **Obligate or strict anaerobe**—grow only in absence of oxygen
- **Facultative anaerobe**—ordinarily aerobes, grow in the presence of oxygen but can also grow in absence of oxygen

3. Carbon Dioxide

Bacteria require small amount of carbon dioxide as well for their growth.

- It is obtained from atmosphere or CO₂ is produced endogenously by bacteria during metabolism
- A few bacteria require additional carbon dioxide (5–10%) for their growth, e.g. *Brucella abortus*, *Neisseria* spp., Pneumococci and are known as **capnophilic bacteria**.

4. Temperature

- Pathogenic bacteria grow best at body temperature, i.e. 37°C
- The optimum temperature is occasionally higher, i.e. 42°C (e.g. for *Campylobacter jejuni*) or lower, i.e. 30°C (e.g. for *Yersinia pestis*)

- On the basis of temperature requirements, bacteria are categorized into three groups:
 - i. **Psychrophiles**—grow optimally below 15°C and are capable of growing at 0°C. Generally do not grow above 20°C. Most of them are soil and water saprophytes
 - ii. **Mesophiles**—grow at moderate temperature. They grow best at temperature 20–40°C. Majority of them are pathogenic organisms
 - iii. **Thermophiles**—grow optimally at temperatures greater than 45°C (range 45–80°C). Most of them are spore forming, e.g. *Bacillus* and *Clostridia*. They live in soil and water. A few of them are pathogenic to humans. Extremely thermophilic bacteria grow at 250°C

5. pH

- The growth and multiplication of bacteria is affected by pH of the medium
- Most pathogenic bacteria grow best (optimum pH) at a neutral or slightly alkaline pH (7.2–7.6)
- Growth is poor below pH 6.0 or above pH 7.8 and stops at pH below 5.0 and pH above 9.0
- Some bacteria grow at acidic pH, e.g. *Lactobacillus* spp. and are known as **acidophilic bacteria**
- *Vibrio cholerae* are sensitive to acid but tolerate alkali

6. Light

- Bacteria grow well in dark.
- They are sensitive to ultraviolet rays and other radiations
- Photosynthetic bacteria require light and photochromogenic mycobacteria produce pigment only when exposed to light

7. Osmotic Effect

- Due to mechanical strength of cell wall, bacteria are able to tolerate osmotic variations but sudden exposure to hypertonic solutions may cause **plasmolysis**, i.e. shrinkage of protoplasm because of osmotic withdrawal of water. It occurs more readily in Gram-negative bacteria than Gram-positive bacteria
- Sudden transfer to distilled water may cause **plasmolysis**, i.e. swelling and rupture of cell because of excessive osmotic water flow inside the cell

■ What is bacterial growth?

Bacterial cells, like other living cells are metabolically active. Bacterial growth means balanced increase in the mass of all cellular constituents. When the growth reaches a critical mass, cell division (binary fission) occurs.

■ Describe the modes of bacterial cell division.

Bacteria multiply by simple binary fission in two equal daughter cells (Fig. 5.1).

- The division starts, when a bacterial cell reaches a critical mass in its cellular constituents
- This occurs in the following way:
 1. The circular double-stranded DNA molecule (nucleus) during replication gets separated into two strands and form new complimentary strand resulting in two identical double stranded DNA molecules, which are distributed into two daughter cells
 2. A transverse septum grows across the cell from the cell membrane
 3. Cell wall material is deposited and the two daughter cells are separated
- Some bacteria also reproduce by **budding** in which a small bud develops from the mother cell and separates and grows further to form a new bacterium
- The time required for a bacterium to form two daughter cells under optimum conditions is known as generation time or population doubling time
- The generation time in most bacteria is 20 minutes, however in tubercle bacilli it is 20 hours and in lepra bacilli it is 20 days

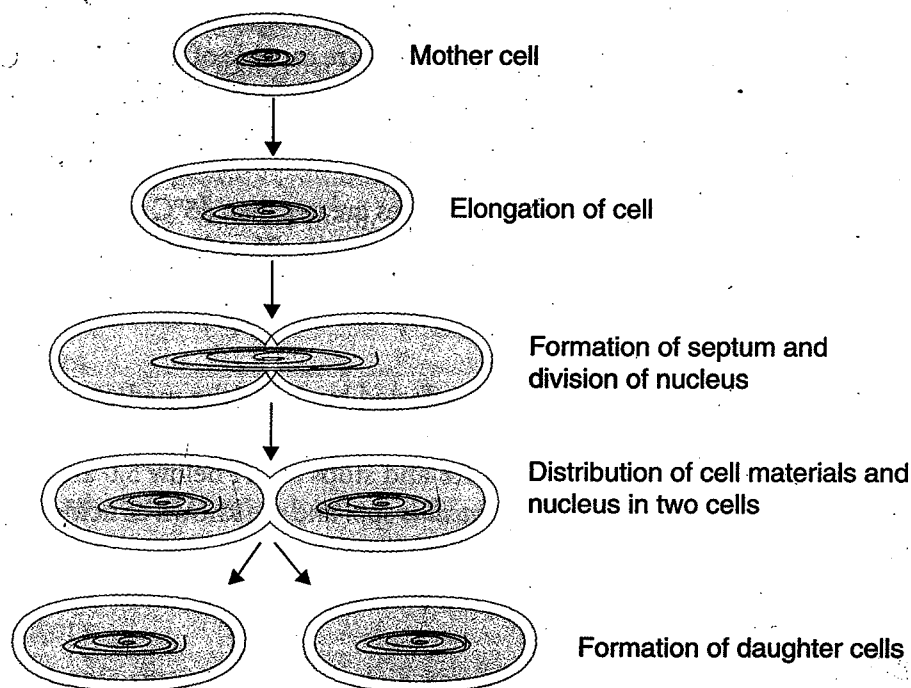


Fig. 5.1 Binary fission in bacteria.

✓ ■ What is bacterial growth curve? Draw and explain its various phases?

When a bacterium is inoculated into suitable liquid medium and incubated at suitable temperature and pH, the culture passes through different phases of growth. When bacterial count of such culture is determined at different intervals and plotted in relation to time, a growth curve having following phases is obtained:

Lag Phase

- Immediately after inoculation—no multiplication occurs
- Organisms adapt to new environment and necessary enzymes and metabolic intermediates are synthesized to reach the stage of multiplication and cell size increases
- This period between inoculation and beginning of multiplication is known as lag phase where no appreciable increase in the number occurs
- It lasts for 1–4 hours based on species of bacterium, nature of medium, temperature and other growth conditions

Log Phase (Exponential Phase)

- Cell division starts at maximum rate
- The number of bacteria increases exponentially with time

Stationary Phase

- The growth rate slows as nutrients are depleted and toxic products are accumulated
- The rate of cell division equals the rate of death. Hence, the viable counts remain stationary

Decline Phase

- The rate of bacterial division is slower than the rate of death
- Results in the decline in total viable count (Fig. 5.2)

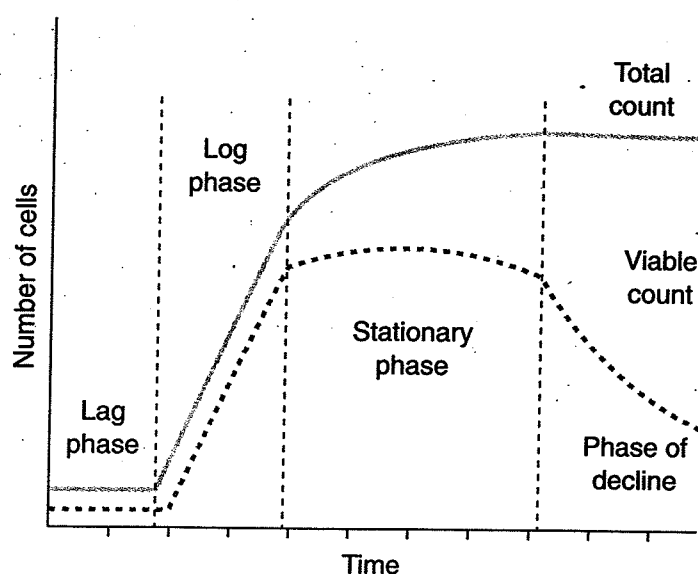


Fig. 5.2 Bacterial growth curve.

Table 5.1 Morphological and physiological alterations of bacteria during different phases of growth

Characteristic alteration	Phase
1. Maximum cell size	Towards the end of lag phase
2. Smaller size and uniform staining	Log phase
3. Variable Gram reaction, irregular staining due to storage granules and sporulation	Stationary phase
4. Secondary metabolic products and involution forms	Phase of decline

- In addition to exhaustion of nutrients and accumulation of toxic products, autolysis may be the cause of decline of number of viable counts and even the total count may be declined
- If the total count is plotted, it runs parallel to the viable count up to the stationary phase, after that it continues steadily without any decline in the number till death due to autolysis starts. After autolysis starts, the total count also shows decline in number
- During the different stages of the growth curve, bacteria show morphological and physiological alterations (Table 5.1)

■ Describe the various methods adopted for measuring bacterial growth.

The bacterial growth can be measured in terms of mass of cellular material or cell numbers. The cell mass can be measured in terms of

- Dry weight
- Packed cell volume
- Nitrogen content
- Turbidity determination by photoelectric colony meter or spectrophotometer. This is the most convenient method.

The cell number can be counted as

- Total count—total number of cells
- Viable count—viable number of cells

Total count

It is a total number of bacteria in a given suspension (including both living and dead organisms). Total number of bacteria can be detected by counting the bacteria under microscope using counting chamber or in a specially designed chambers such as Coulter counter.

Viable count

It is the number of living (viable) bacteria (excludes dead bacteria).

- Viable number of bacteria can be detected by dilution method. This is the commonly used method, but it does not give accurate count, e.g. most probable number (MPN) test for finding out presumptive coliform count in drinking water
- Plating method, in which appropriate dilutions of specimen are inoculated on to solid medium or as pour plates. Based on the number of colonies, total number of bacteria in a given suspension is estimated (one colony is formed by one bacterium, therefore number of colonies formed gives the viable count)

■ What is continuous culture?

When bacteria are grown in a vessel of liquid media (batch culture), after sometime the cell division comes to halt and multiplication of cell is stopped due to depletion of nutrients and/or accumulation of toxic products. By the use of special devices for replenishing nutrients and removing bacterial cells (chemostat or turbidostat), continuous culture of bacteria is maintained for research and industrial purposes.

■ How is *in vivo* growth of bacteria different from *in vitro* growth?

There is a significant difference in bacterial growth *in vivo* (human body) and *in vitro* (artificial culture medium). *In vivo*, when bacteria multiply in host tissue, the situation may be intermediate between a batch culture and continuous culture; the sources of nutrients may be adequate but the bacterium has to protect itself from the defense mechanisms of the body. The growth *in vivo* is influenced by the various defense mechanisms.

■ What do you understand by 'bacterial metabolism'? Describe in short (a) oxidation, (b) fermentation, and (c) redox potential in reference to bacterial metabolism.

The process of absorption of food material (substances such as carbohydrates, proteins or fats), their breakdown and utilization within the bacterial cell and elimination of metabolic end products are carried out by certain fundamental metabolic pathways. Bacteria differ in the way in which the energy source is broken down to produce energy. Aerobic bacteria obtain their energy by oxidation involving oxygen as the ultimate hydrogen (electron) acceptor, however the hydrogen acceptor is other than oxygen in anaerobic bacteria. Facultative anaerobes utilize both the pathways.

(a) Oxidation

In aerobic bacteria, where the ultimate electron acceptor is oxygen, the carbon and energy source may be completely oxidized to carbon dioxide and water. During this process, energy is generated by a process called **oxidative phosphorylation**, i.e. by the production of energy rich phosphate bonds and their transfer to adenosine diphosphate (ADP) forming adenosine triphosphate (ATP).

(b) Fermentation

In anaerobic bacteria, the electron acceptors are nitrates or sulphites. In these bacteria, anaerobic growth occurs by a process in which carbon and energy source act as both electron

donor and electron acceptor in a series of oxidoreductions. This process is known as fermentation and it leads to formation of acids (lactic acid, formic acid, pyruvic acid), alcohols and gases (hydrogen or carbon dioxide). During the process of fermentation, energy rich phosphate bonds are produced by the introduction of organic phosphate into intermediate metabolites. This process is known as **substrate level phosphorylation**. These energy rich phosphate bonds are transferred on to ADP to form ATP. This process of fermentation is carried out by both obligate and facultative anaerobes. However, facultative anaerobes may obtain their energy either by fermentation or by respiration. The amount of energy produced under anaerobic conditions is comparatively less and hence the growth of facultative anaerobe is comparatively much more in aerobic conditions, as in the presence of oxygen they derive energy by respiration.

(c) Redox Potential (Oxidation-Reduction Potential)

Oxidizing agents are substances capable of accepting electrons, and **reducing agents** are substances that are able to part with electrons.

The capability of substance to accept or lose the electron is called oxidation-reduction potential or redox potential (Eh). Eh is measured in millivolts. It is higher in oxidized substances (oxidized system) and lower in reducing agents (reduced system). The strict anaerobes require low redox potential—less than 0.2 volt, however, the redox potential of most of the media exposed to air is +0.2 to 0.4 volt at pH 7.0.

6

Chapter

Classification of Bacteria

- Define the words taxonomy and classification with reference to bacteria.

The art of biological classification is known as Taxonomy. It is concerned with the classification or systematic arrangement of bacteria into groups or categories called taxa (singular is taxon). Classification is orderly arrangement of units into groups or larger units.

- Name the three taxonomic kingdoms into which organisms are categorized. Of these, to which one do microorganisms belong.

Organisms are divided into three main kingdoms:

1. Animals
2. Plants
3. Protista

Kingdom **Protista** contains microorganisms including eukaryotes and prokaryotes (bacteria).

- Which is the commonly used phylogenetic system of bacterial classification. Give one example.

It has been published as **Bergey's Manual**. There is no universally accepted bacterial classification. Bergey's Manual of Systematic Bacteriology—International classification founded by D. Bergey—is commonly used in Microbiology. According to this, all lower organisms are divided as Divisions, Classes, Orders, Families, Tribes, Genera and Species. Major groups are differentiated based on important characters such as Gram reaction, spore formation, etc. The genus and species are differentiated based on less important properties such as fermentation reactions, nutritional requirements, etc.

The full taxonomical position of *Salmonella typhi* is as follows:

- Division: Protophyta
- Class: Schizomycetes
- Order: Eubacteriales
- Family: Enterobacteriaceae
- Tribe: Salmonellae
- Genus: *Salmonella*
- Species: *Salmonella typhi*

- Based on *Bergey's Manual of Systematic Bacteriology* classify pathogenic bacteria, starting from Division Protophyta till the level of Genus.

According to Bergey's Manual, all lower microorganisms of the plant kingdom, Division—Protophyta are subdivided into three classes as:

Class I: Schizophyceae

This consists of blue-green algae which are not pathogenic to man and animals.

Class II: Schizomycetes

These are the microorganisms lacking chlorophyll. This class includes 10 orders, out of which following five orders are pathogenic to man and animals.

Order I

Pseudomonadales—*motile by polar flagella or nonmotile*

Family: Spirillaceae, Genus: *Vibrio*, *Spirillum*, etc.

Order IV

Eubacteriales—*true bacteria, rod-shaped, coccoid-shaped, motile by peritrichate flagella or nonmotile*

- (a) Family: Enterobacteriaceae, Genus: *Escherichia*, *Klebsiella*, *Proteus*, *Salmonella*, *Shigella*, etc.
- (b) Family: Brucellaceae, Genus: *Brucella*, *Haemophilus*, *Bordetella*, *Pasteurella*, etc.
- (c) Family: Bacteroidaceae, Genus: *Bacteroides*, *Fusobacterium*
- (d) Family: Micrococcaceae, Genus: *Micrococcus*, *Staphylococcus*, *Peptococcus*, etc.
- (e) Family: Neisseriaceae, Genus: *Neisseria*
- (f) Family: Lactobacillaceae, Genus: *Diplococcus*, *Streptococcus*, *Peptostreptococcus*, etc.
- (g) Family: Corynebacteriaceae, Genus: *Corynebacterium*
- (h) Family: Bacillaceae, Genus: *Bacillus*, *Clostridium*

Order V

Actinomycetales—*filamentous, branching cells*

- (a) Family: Mycobacteriaceae, Genus: *Mycobacterium*
- (b) Family: Actinomycetaceae, Genus: *Actinomyces*, *Nocardia*

Order IX

Spirochaetales—*nonrigid, spiral forms*

- (a) Family: Spirochaetaceae, Genus: *Borrelia*, *Treponema*
- (b) Family: Leptospiraceae, Genus: *Leptospira*

Order X

Mycoplasmatales—*small, pleomorphic, filterable, microorganisms, e.g. Mycoplasma*

Family: Mycoplasmataceae, Genus: *Mycoplasma* and *Ureaplasma*

Class III: Microtobiotes

This class includes small, filterable microbes such as *Rickettsia*, *Chlamydia*, viruses

Family: Rickettsiaceae, Genus: *Rickettsia*, *Orientia*, *Coxiella* and *Ehrlichia*

Order

Chlamydiales

Family: Chlamydiaceae, Genus: *Chlamydia*

■ Enumerate the factors generally considered for identification and classification of bacteria.

For identification and classification of bacteria following important characters are selected:

- Morphology
- Staining
- Cultural characters
- Biochemical reactions

- Antigenic structure
- Guanine: Cytosine ratio of DNA

■ Give nomenclature of a bacterium.

Each bacterium possesses two names—a generic name and a specific name

- **Generic name:** It is usually a Latin noun that begins with an initial capital
- **Species name:** It begins with an initial small letter even though it is a name of a person or place and indicates one of the following things:
 - some property, e.g. *albus* meaning white
 - the disease it causes, e.g. *cholerae* from cholera
 - the person who discovered, e.g. *welchii* after Welch
 - the animal in which it is found, e.g. *suis* meaning pig
- The generic and specific names should be *italicized*, i.e. they should be written in italics or underlined.

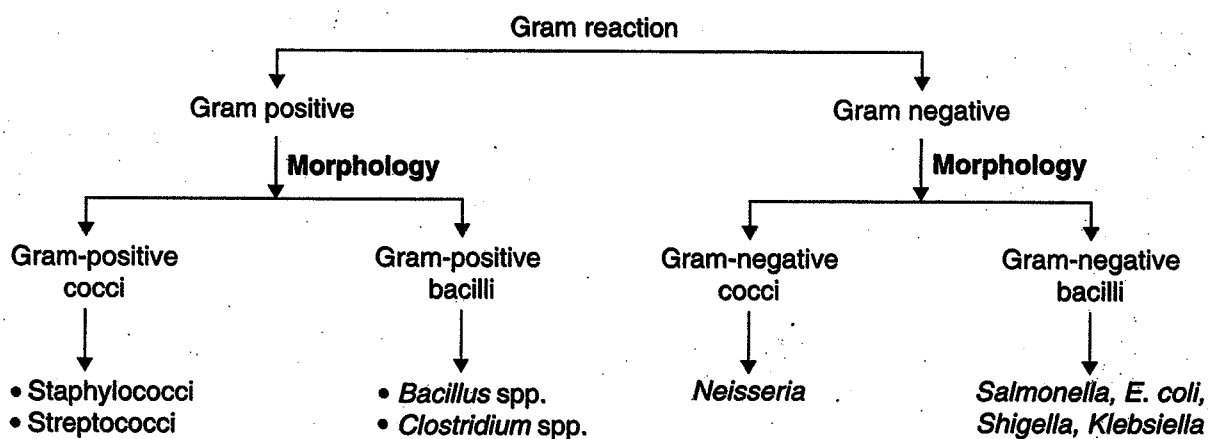
■ Classify bacteria on the basis of morphology.

Based on their morphology bacteria are classified into two types:

1. **Lower bacteria:** They are simple, unicellular microorganisms. Depending on their shape they can further be grouped into cocci, coccobacilli, bacilli, vibrio, spirilla and spirochaetes (Fig. 4.1).
2. **Higher bacteria:** They are filamentous organisms, e.g. Actinomycetes—branching filamentous bacteria

■ Classify bacteria based on their Gram staining characters.

Based on Gram staining reactions bacteria are classified as follows:



■ Classify bacteria based on acid-fast staining reactions.

Based on the acid-fast staining reaction (some bacteria appear bright red colour others do not), they are grouped into the following two types:

1. Acid-fast bacilli—e.g. tubercle and leprosy bacilli
2. Nonacid fast bacilli—Bacteria other than acid-fast bacilli are nonacid-fast bacilli, e.g. Staphylococci, *E. coli*, *Salmonella*, etc.

■ **Classify bacteria on the basis of the arrangement of their cells.**

Based on characteristic arrangement of their cells, bacteria are distinguished into diplococci, streptococci, staphylococci, tetrad, sarcina, streptobacilli, diplobacilli, Chinese letter arrangement, etc. (see Fig. 4.2).

■ **Very briefly describe Adansonian classification, genetic classification and intraspecies classification.**

Adansonian Classification

In this system of classification, all the characters (similar and different characters) are taken into account with equal importance and organisms are grouped on the basis of similarities in large number of characters.

Genetic Classification

It is based on the degree of genetic relatedness of different bacteria. This system is most natural or fundamental as all properties of bacteria are regulated by genes. Composition of DNA is the basis for this classification. By estimating G+C (guanine and cytosine) content of DNA, bacteria are classified.

Intraspecies Classification

For diagnostic or epidemiological purposes, bacterial species are subclassified as

- Biotypes, based on biochemical properties
- Serotypes, based on antigenic structure
- Phage types, based on bacteriophage susceptibility
- Colicin types, based on production of bacteriocins

A species may be divided first into groups and then into subtypes.

7

Chapter

Culture Media

■ Highlight the importance of culture technique in the study of microorganisms.

It is not always possible to identify the microorganisms by the microscopic methods alone. Because of their similarity in morphology and staining characters, it is necessary to grow them on suitable artificial culture medium to identify them. This process of growing organisms helps in obtaining organisms in pure form (isolation) and in undertaking further testing to confirm their identity.

■ What is a culture medium?

The food material on which the organism is grown is known as **culture medium** and the growth of organism is known as **culture**. The culture medium provides nutrients necessary for growth and fulfills other requirements such as pH, oxygen, temperature, etc. required for optimum growth of the organism.

■ What are the characteristics of an ideal culture medium?

Following are the characteristics of an ideal culture medium:

- Satisfactory growth from small inoculum—even from single cell
- Rapid growth
- Cheap and easy to prepare
- Easily reproducible
- Demonstrate all the characteristics in which we are interested

■ What are the basic requirements of culture media?

The basic requirements of culture media are:

- Energy source
- Carbon source
- Nitrogen source
- Salts such as sulphates, phosphates, chlorides and carbonates of K, Na, Mg, Ca, Fe and trace elements such as Mn, Mo, Cu
- pH—a satisfactory pH
- Adequate oxidation-reduction potential (Eh)
- Growth factors such as tryptophan for *Salmonella typhi*, glutathione for Gonococci

■ What are the common ingredients of culture media and how are they helpful in growth of bacteria?

The common ingredients of culture media and their usefulness in growth of bacteria are as follows:

1. Water

- Essential for existence of living cells

- Source of hydrogen and oxygen
- Demineralized distilled water is used

2. Peptones

- Golden granular hygroscopic powder—obtained from meat, casein fibrin or soya flour
- Complex mixture of partially digested proteins—containing proteoses, amino acids, polypeptides, phosphates, minerals (K, Mg, Ca, Fe, Zn) and accessory growth factors (riboflavin, nicotinic acid)
- *Functions:* Nitrogen source, carbon source, buffers

3. Meat Extract

- Extract of meat obtained by special procedure, commercially available as 'Lab Lemco'
- Contains protein degradation products, carbohydrates, inorganic salts, enzyme excitors and growth factors that are rich in vitamin B complex
- *Functions:* Source of growth factors, inorganic salts, etc.

4. Yeast Extract

- Extract of yeast cells
- Contains proteins, amino acids, growth factors (vitamin B), carbohydrates, and inorganic salts like potassium and phosphates
- *Functions:* Source of growth factors and hence excellent stimulator of growth. It can be used as a substitute for meat extract

5. Electrolyte

- Sodium chloride or other electrolytes
- *Function:* Essential to maintain osmotic pressure

6. Agar

- Dried mucilaginous substance obtained from seaweeds
- Available as long shreds or in powder form
- Contains mainly long chain polysaccharides, protein-like material and inorganic salts
- Does not provide any nutrition to growing organisms
- *Function:* It melts at 98°C and generally solidifies at 42°C—hence used as a solidifying agent
- Differs in properties from manufacturer-to-manufacturer, batch-to-batch variations also exist, e.g.
 - i. Japanese agar—yields gel at a concentration of 2%
 - ii. New Zealand agar—yields gel at a concentration of 1%

■ **Write short notes on various types of culture media, classified based on their physical state.**

The various types of media used for culturing are:

1. Liquid Media

Liquid in nature, does not exhibit specific characteristics on the basis of which identification can be made, and also does not permit isolation of different types of bacteria from mixed populations

Uses

They can be used for:

1. Further testing of pure culture of bacteria
2. Preparing bulk cultures for preparation of antigens or vaccines
3. Obtaining growth of organisms from blood or water when large volumes have to be tested

2. Solid Media

Solid in nature, produce discrete visible growth.

Uses

1. To obtain a pure growth
2. To study distinctive colony morphology and other characteristic features, e.g. pigmentation, haemolysis, etc.

3. Semi-solid Media

This type of media is neither liquid nor solid. It is in a phase between liquid and solid.

Uses

1. Cultivation of anaerobic and microaerophilic organisms
2. For studying bacterial motility

■ Write short notes on various types of culture media based on their nutritional factors.

Based on nutritional factors, the following six types of culture media are known:

1. Simple media
2. Complex media
3. Synthetic media
4. Semidefined media
5. Nonsynthetic media
6. Special media

Simple Media (Basal Media)

Media with relatively simple chemical composition, e.g. nutrient broth and peptone water are the simplest liquid media and form the basis of all other laboratory media. Addition of 2–3% agar to nutrient broth makes it nutrient agar, which is the simplest solid basal medium, routinely used for diagnostic purpose (Fig. 7.1). Addition of 0.2–0.4% agar makes it semi-solid medium used for the study of bacterial motility. Addition of 6% agar makes it firm agar that prevents spreading or swarming of growth of bacteria such as *Proteus*.

Complex Media

These are the media with complex composition containing additional ingredients for bringing out certain properties or providing special nutrients required for the growth. All media other than simple media are complex media.

Synthetic or Defined Media

These are the media prepared by using pure chemicals of known chemical composition, hence the exact chemical composition of the medium is known. These are used for special studies, e.g. to determine the nutritional requirements of organism. Dubos medium with Tween 80 is an example.

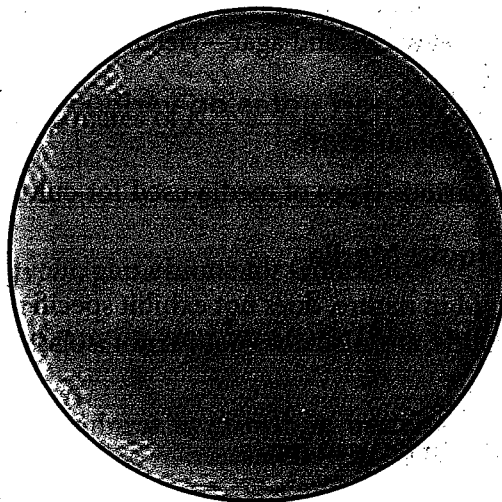


Fig. 7.1 Nutrient agar.

Semi-defined Media

These are media with chemical composition approximately known, e.g. peptone water.

Nonsynthetic Media

These are the media prepared by using compounds of unknown chemical composition, e.g. beef extract, yeast extract, peptones, blood, serum, etc.

Special Media

These are media that are used for special purposes. The special media generally used are:

- Enriched media
- Enrichment media
- Selective media
- Differential media
- Indicator media
- Transport media
- Sugar media

Enriched Media

Basal medium added with substances such as blood, serum or egg is known as enriched medium.

Uses

For growing organisms which are more exacting in their nutritional requirements.

Examples

1. Blood agar—enriched with blood (Fig. 7.2)
2. Chocolate agar—enriched with heated blood (Fig. 7.3)
3. Loeffler's serum slope—enriched with serum (Fig. 7.4)
4. Lowenstein-Jensen (L-J) medium—enriched with egg (Fig. 7.5)

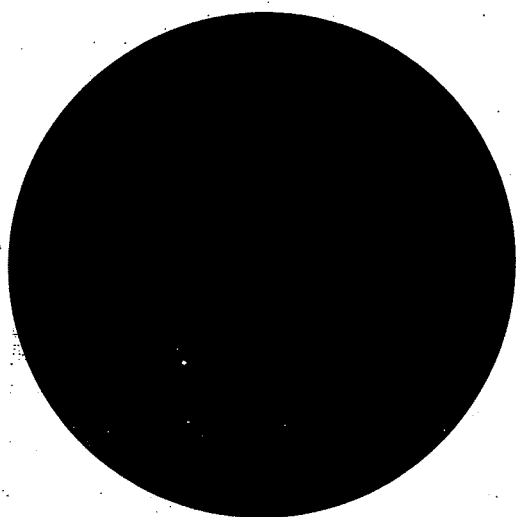


Fig. 7.2 Blood agar.

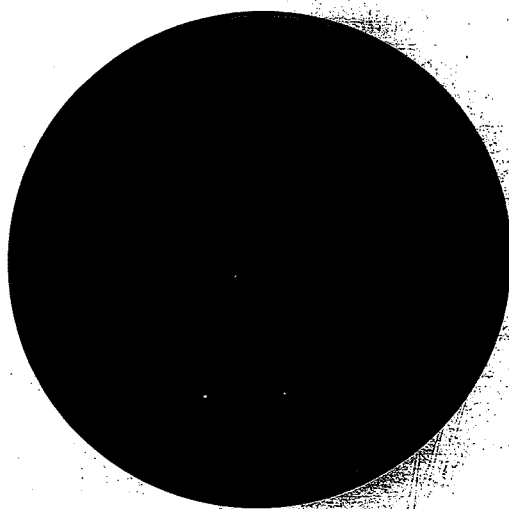


Fig. 7.3 Chocolate agar.

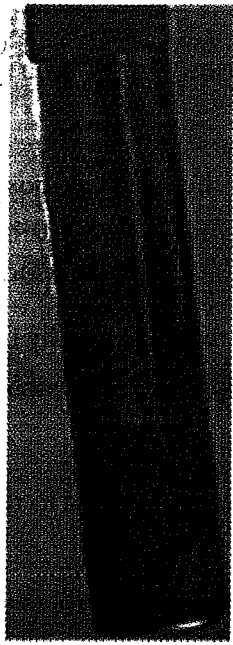


Fig. 7.4 Loeffler's serum slope.

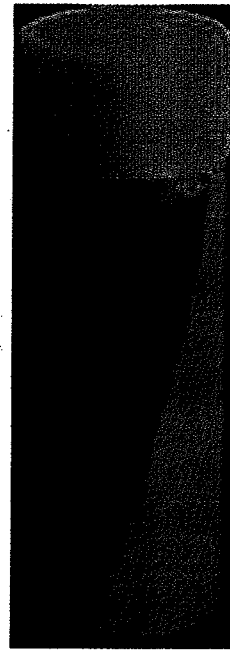


Fig. 7.5 Lowenstein-Jensen medium.

Enrichment Media

- These are liquid media incorporated with substances, which stimulate the growth of wanted organisms or inhibit the growth of unwanted organisms from mixed inocula, such as specimen of faeces in enteric fever
- They selectively favour the growth of organism of interest inhibiting growth of other organisms in a mixed population

Uses

Useful in situations where nonpathogenic or commensal bacteria tend to overgrow the pathogenic ones.

Examples

Tetrathionate broth and selenite F broth inhibit the growth of coliforms in faeces and allow the growth of *Salmonella* spp.

Selective Media

- These are solid media incorporated with inhibitory substances, which inhibit the growth of a large number of organisms and selectively permit the growth of required organism in a greater number
- Selectively facilitate isolation of a particular organism

Uses

To isolate a particular organism from a specimen in which mixed microbial flora is expected.

Examples

1. Deoxycholate citrate agar (DCA) for *Shigella*, *Salmonella*
2. Bile salt agar for *V. cholerae*
3. Wilson-Blair medium for *Salmonella* (Fig. 7.6)
4. Thiosulfate-citrate-bile salts-sucrose (TCBS) agar (Fig. 7.7)



Fig. 7.6 Wilson-Blair medium.

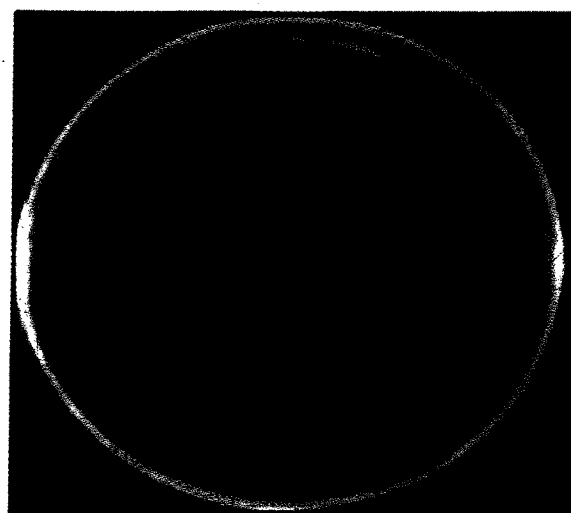


Fig. 7.7 TCBS agar.

Differential Media

- These are media, which differentiate two types of organisms
- These media are incorporated with substances, which help to distinguish organisms based on their characteristic features

Uses

To differentiate two types of organisms.

Examples

MacConkey's medium containing peptone, lactose, sodium taurocholate, neutral red and agar. It differentiates lactose fermenters (which form pink colonies) from nonlactose fermenters (which form colourless or pale colonies) (Fig. 7.8)

Indicator Media

- These are media, which indicate the presence of particular organism by giving particular colour to the colony of organism grown
- These media are incorporated with an indicator which changes colour of organism when it grows

Examples

1. Wilson and Blair medium containing sulphite, which is reduced to sulphide by *Salmonella typhi* producing black colonies
2. MacConkey's medium is also an indicator medium as it forms pink colonies in the presence of neutral red indicator and indicates presence of lactose-fermenting organisms
3. The blood agar (enriched medium) showing haemolysis indicates presence of haemolytic organisms, hence it is also an indicator medium

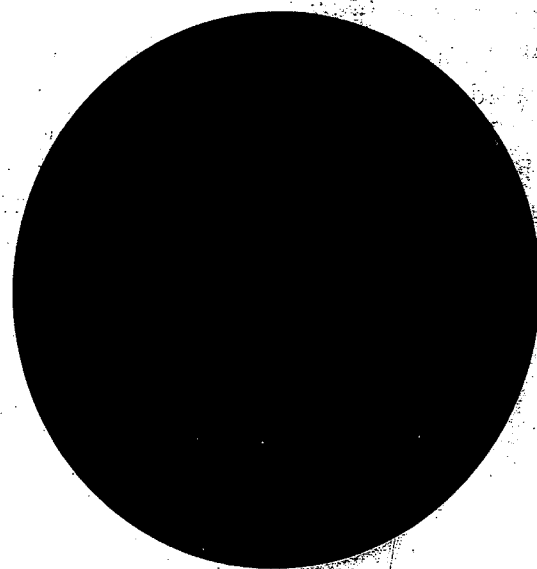


Fig. 7.8 MacConkey's agar.

Transport Media

- Delicate organisms like *Gonococci*, *V. cholerae*, etc. do not survive outside the body for long duration. These organisms may be killed during transportation of the specimen to laboratory if not transported immediately or they may be overgrown by nonpathogenic organisms (e.g. *V. cholerae*)
- To avoid death of delicate organisms during transportation from hospital to laboratory and to avoid overgrowth of nonpathogenic organisms, special media are used for transport of specimens. These media are termed transport media

Examples

1. Stuart's transport medium. It is a non-nutrient soft agar gel containing a reducing agent to prevent oxidation and charcoal to neutralize bacterial inhibitors. It is used for transporting *Gonococci* and other bacteria
2. Buffered glycerol saline transport medium for enteric bacteria
3. Cary-Blair transport medium

Sugar Media

- Media containing fermentable substances (sugars) are known as sugar media
- These media contain 1% sugar (glucose, lactose, sucrose, mannitol, etc.) in peptone water with Andrade's indicator and inverted small tube, the Durham's tube for gas collection (Fig. 7.9)

Uses

Fermentation of sugars results in the formation of (a) an acid that renders pink colour to originally colourless medium, and (b) gas which is indicated by gas bubbles that accumulate in Durham's tube. These fermentation reactions help in identification of the organism.

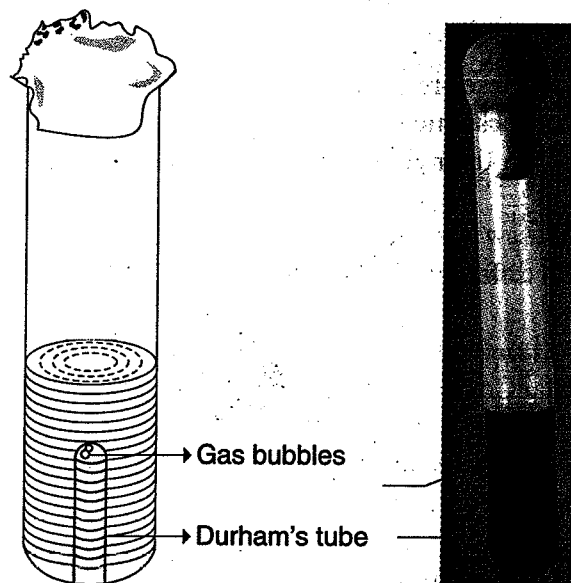


Fig. 7.9 Sugar medium.

Anaerobic Media

Media used for the growth of anaerobic bacteria, e.g. Robertson's cooked meat medium, are called anaerobic media.

The different media can be prepared in laboratory by using basic ingredients. Alternatively, they can be prepared by using commercially available readymade dehydrated media, which is an easier method. Qualitywise there is uniformity and no batch-to-batch variation as commonly seen with media prepared using basic ingredients.

8

Chapter

Culture Methods

■ What do you understand by 'culture methods'?

The methods used for growing microorganisms are known as culture methods. Different culture methods are available for culture of bacteria, based on the purpose for which they are intended.

■ Enumerate the indications for culture of bacteria in a clinical laboratory.

In a clinical laboratory, following are the indications for culture:

- To isolate bacteria in pure culture from the clinical specimens and to identify them
- To demonstrate their properties
- To obtain sufficient growth for antigen preparation
- To study susceptibility of bacteria to antibacterial agents, bacteriophages, bacteriocins, etc.
- To estimate viable counts
- To maintain stock cultures

■ What do you understand by 'inoculation' of culture media? Describe the types of equipment used for this purpose.

- It is the process of insemination of bacteria in culture media. It may be done from
 - Pathological material (primary culture) for isolation of pathogenic organism or
 - Another culture (subculture) for the further study of the isolated organism
- Inoculation loop, spud, straight wire or Pasteur pipette is used for inoculating the media

Inoculation Loop

It is a platinum wire loop of 23 SWG (standard wire gauge) thickness or nichrome wire loop of 24 SWG about 3" long held on a metal holder or fused into a glass rod. The free end of wire is bent in the form of a loop of about 2–4 mm in diameter. Nichrome wire is cheap but takes more time to cool after flaming (Fig. 8.1a).

Uses

1. To inoculate culture media
2. For preparation of smear
3. For collection of specimens

Spud

It is a straight wire bent at an angle and the tip is flattened with a hammer (Fig. 8.1b).

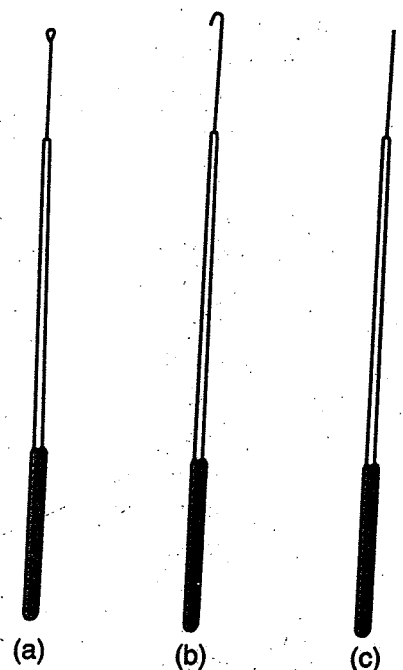


Fig. 8.1 Inoculation loop (a), spud (b) and straight wire (c).

Uses

For scrapping medium to pick up sticky colonies.

Straight Wire

It is a straight wire, bent neither like loop nor like spud (Fig. 8.1c).

Uses

1. For stab or shake culture
2. To pick up exactly a single colony from mixture

Pasteur Pipette

It is a capillary pipette with rubber teat.

Uses

For inoculating pathological fluids like urine, cerebrospinal fluid and liquid cultures.

■ **Describe the various methods adopted for culture of bacteria.**

The different methods of culture used in the laboratory are described in the following text.

1. Streak Culture

- A loopful of material (clinical specimen) is taken with the help of flame sterilized and cooled loop and placed on the surface of dried culture plate near the peripheral area. This is known as **primary inoculum**
- This primary inoculum is then spread thinly over the plate by streaking it in a series of parallel lines in different segment of the plate
- The loop used for spreading should be flamed and cooled in between the different sets of streak
- On incubation, growth may be confluent at the site of primary inoculum. The growth becomes progressively thinner and well-separated colonies are formed over the final series of streaks (Fig. 8.2)

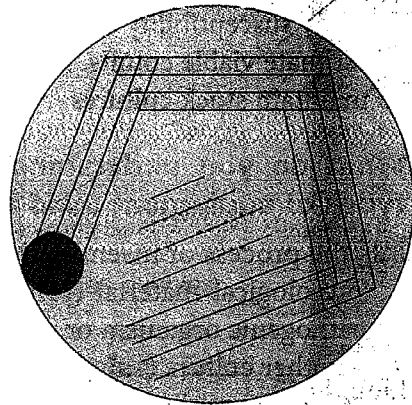


Fig. 8.2 Streak culture method.

Uses

For the isolation of bacteria in pure culture from clinical specimens.

2. Lawn or Carpet Culture

- The surface of the plate is flooded with a liquid culture or a suspension of the bacterium and excess inoculum is pipetted off, or
- The surface of the plate is inoculated with the help of swab soaked in the bacterial culture or suspension, alternatively one or two drops of bacterial culture or suspension is placed on the surface of the plate with the help of pipette, then the pipette is sealed and bent at the tip and used to spread the material.

Uses

1. For antibiotic susceptibility testing by disc diffusion method
2. For bacteriophage typing
3. For preparing bacterial antigens and vaccines where a large amount of bacterial growth is required

3. Stroke Culture

It is made in tubes containing agar slope (slant), nutrient agar is most commonly used.

Uses

To obtain a pure growth of bacterium for slide agglutination and other diagnostic tests.

4. Stab Culture

Culture is made by puncturing deep inside the agar with the help of straight wire charged with culture material.

Uses

1. To demonstrate gelatin liquefaction
2. To demonstrate oxygen requirement of bacteria
3. To maintain stock cultures of bacteria for preservation of bacteria

5. Pour Plate Culture

- The inoculum to be tested is serially diluted
- Known amount (1 ml) of each dilution is mixed with 15 ml of melted agar at 45–50°C and mixed well
- The contents of tube are poured in a sterile Petri plate and allowed to set
- After overnight incubation at 37°C, the colonies distributed throughout the depth of media are counted. The number gives the viable bacterial count in a given suspension

Uses

1. To quantitate bacteria in urine cultures
2. For analysis of water
3. For assay of antibiotics, enzymes, etc.

6. Liquid Culture

- Liquid cultures in tubes, screw capped bottles or flasks are inoculated with loop, pipettes or syringes
- Liquid cultures are preferred when large and quick yield is required

Uses

1. For blood culture and sterility tests, where the number of bacteria in the inoculum are expected to be small
2. For specimens containing antibiotics and other antibacterial substances, which are rendered ineffective by dilution in the medium

Disadvantages

1. It does not provide pure culture from mixed inoculation
2. Identification of bacteria is not possible

■ Which temperature is best suited for incubating pathogenic bacteria?

Most of the pathogenic microorganisms are mesophilic and grow best at 37°C, i.e. body temperature of human beings is the optimum temperature for their growth. Hence, the inoculated culture media are incubated at 37°C in an incubator. Incubator is structurally similar to hot air oven (Fig. 8.3).

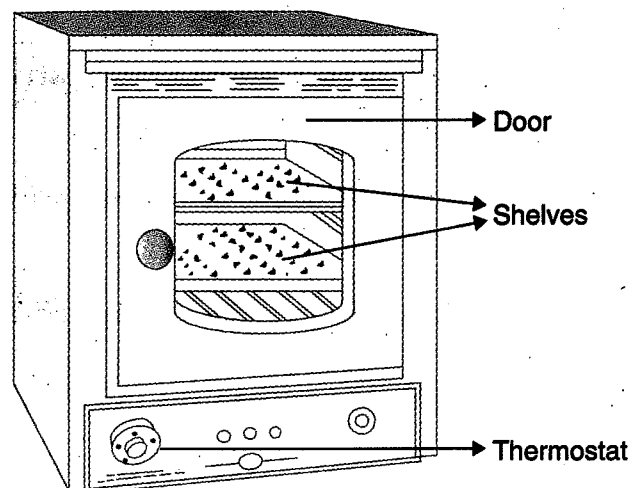


Fig. 8.3 Bacteriological incubator.

■ **Mention the characteristic growth condition for anaerobic bacteria. Describe the various methods adopted for creating anaerobic conditions to culture anaerobic bacteria.**

- Anaerobic bacteria do not require oxygen for their growth. They grow in the absence of oxygen
- The anaerobic conditions or anaerobiosis can be achieved by the following methods:

1. Production of Vacuum

- This is done by incubating cultures in a **vacuum desiccator**.
- Results are not satisfactory, hence this method is not used

2. Displacement of Oxygen

- Oxygen is displaced by **inert gases** like hydrogen, nitrogen, helium or carbon dioxide
- Complete anaerobiosis is not achieved by this method, hence it is ineffective and not generally used
 - **Candle jar** is the most popular method. In this, inoculated plates are placed in a large airtight container and a lighted candle is kept before the lid is sealed. It is expected that burning candle will utilize all the oxygen before it gets extinguished, but some oxygen is always left behind, hence it is ineffective as a method of anaerobiosis. However, it provides 5–10% CO_2 concentration condition, which is suitable for the growth of capnophilic bacteria (Fig. 8.4)

3. Displacement and Combustion of Oxygen

- For this, **McIntosh and Fildes jar** is used
- It is the most reliable and widely used method

Structure

- It consists of a glass or metal jar with a metal lid, which can be clamped airtight with screw
- The lid has two tubes with taps—one acting as inlet for introduction of gas and the other as an outlet
- The lid also has two terminals, which can be connected to electric supply and a catalyst (alumina pellets coated with palladium) under the lid
- It also has pressure gauze (Fig. 8.5)

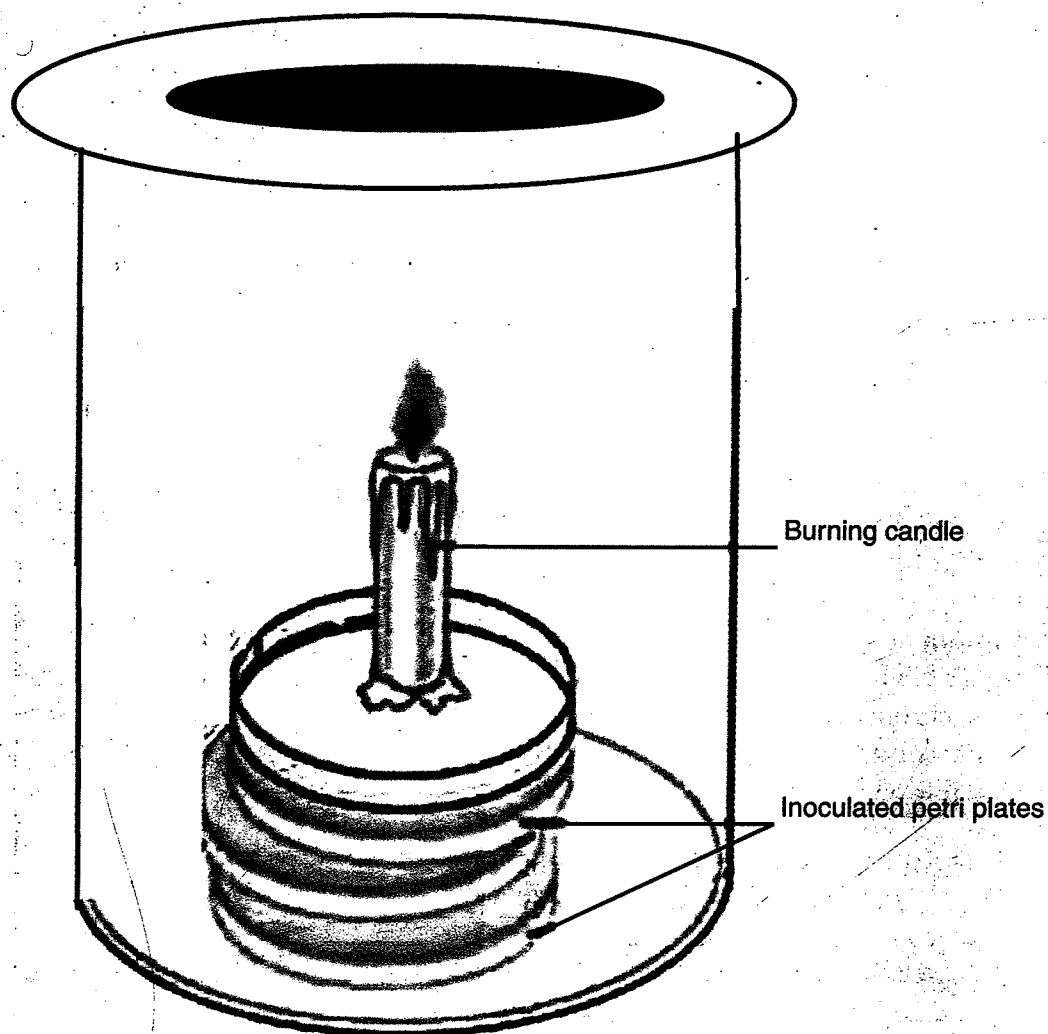


Fig. 8.4 Candle jar.

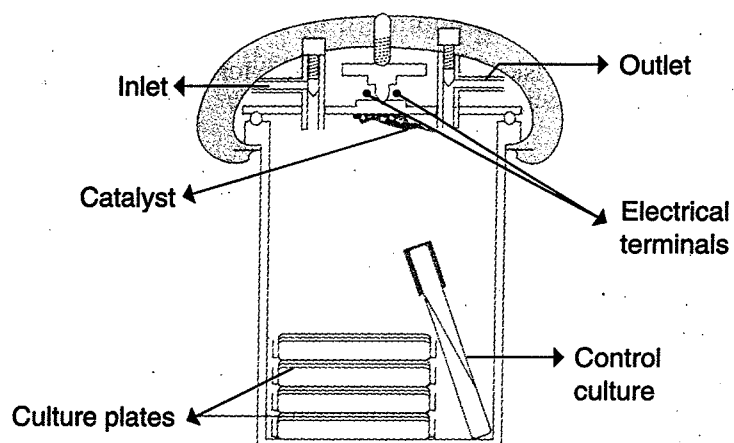


Fig. 8.5 McIntosh and Fildes jar.

Operation

- * Inoculated culture plates are placed inside the jar and the lid is clamped
- * The outlet is connected to vacuum pump and the air inside is evacuated
- * The outlet is closed and hydrogen is passed through inlet till it is filled (monitored by pressure gauge)

- Electric terminals are switched on to heat the catalyst, which catalyzes the reaction between hydrogen and residual oxygen to form water
- Alternatively, room temperature catalyst can be used, which need not be heated.
- An indicator should be kept inside the jar to confirm whether anaerobiosis is achieved or not
- Two types of indicators can be used:
 - **Chemical indicator:** Reduced methylene blue, which is colourless under anaerobic conditions but turns blue on exposure to oxygen
 - **Biological indicator:** A plate inoculated with culture of strict aerobe, e.g. *Pseudomonas aeruginosa* is placed in jar—no growth confirms anaerobiosis

4. Absorption of Oxygen by Chemical or Biological Methods

Chemical Methods

1. **Pyrogallol:** In this method, a tube containing pyrogallol acid and sodium hydroxide is placed inside an airtight jar that absorbs oxygen and produces anaerobic conditions
2. **Chromium and sulphuric acid:** These two chemicals react in the presence of oxygen forming chromous sulphate and produce anaerobic conditions
3. **GasPak (Fig. 8.6)**
 - a. Presently, it is the method of choice
 - b. The GasPak packet containing pellets of citric acid, sodium bicarbonate, sodium borohydrate and cobalt chloride is commercially available
 - c. It generates hydrogen and carbon dioxide on addition of water
 - d. Hydrogen combines with oxygen to produce water and creates anaerobic conditions
 - e. **Working:** Inoculated plates are placed in airtight jar, the packet of GasPak with water is added and the lid is tightly closed
 - f. **Advantages:** Simple, reliable, no risk of explosion as in McIntosh–Fildes jar

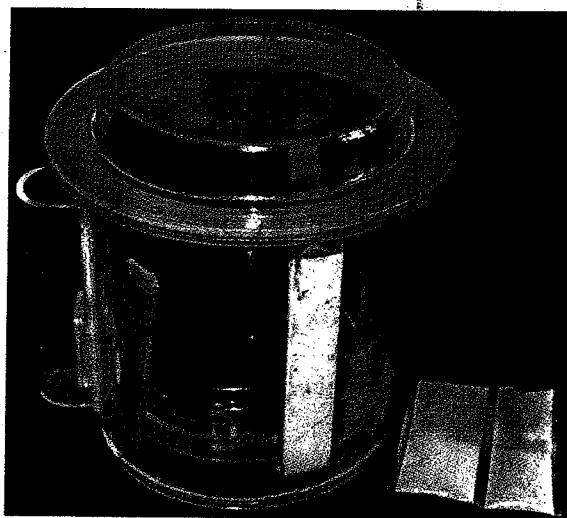


Fig. 8.6 GasPak jar.

Biological Methods

- By incubating the culture of aerobic organisms such as *Pseudomonas aeruginosa* in airtight jar with assumption that utilization of oxygen by aerobic bacteria for their growth creates anaerobic condition
- This method is slow and ineffective

5. By Using Reducing Agents

Oxygen in a medium can be reduced by using various reducing agents such as:

- 1% glucose
- 0.1% thioglycollate
- 0.1% ascorbic acid
- 0.05% cysteine
- Cooked meat pieces
- Flamed red-hot iron pieces

Robertson's Cooked Meat Medium (RCM) (Fig. 8.7)

- It is the most widely used medium for anaerobes.
- It contains fat free minced cooked meat of an ox's heart in nutrient broth
- The meat particles contain unsaturated fatty acids, which utilize oxygen for their oxidation, and the reducing substances such as glutathione and cysteine utilize oxygen and help in the growth of anaerobes
- The heating of medium before inoculation at 80°C for 30 minutes makes it oxygen-free and layering with sterile liquid paraffin after inoculation prevents the fresh entry of oxygen into the medium

■ **Mention the various methods of obtaining pure cultures.**

The following methods can be used for obtaining pure cultures:

- **Surface plating**—is routinely used. This leads to formation of isolated colonies of different bacteria, which may be picked out for further identification and study
- Use of **enriched, selective and indicator media** for isolation of pathogens from specimens with varied flora such as faeces
- Pure cultures may be obtained by **pretreatment of specimens with bactericidal substances**, which destroy unwanted bacteria without producing any harm to wanted bacteria, e.g. treatment of sputum with alkali and acid destroys most commensals and help to get pure culture of *Mycobacterium tuberculosis*
- Pure cultures of obligate aerobes and anaerobes can be obtained by **incubating under aerobic and anaerobic conditions** respectively
- Pure cultures of spore formers can be obtained by **heating mixture at 80°C**. Heating eliminates vegetative forms of bacteria
- Bacteria can also be separated by **incubating at a different temperature**, e.g. pure culture of thermophiles like clostridia can be obtained by incubating them at 60°C
- Motile bacteria can be separated from nonmotile bacteria by **Craigie's tube or U tube technique**

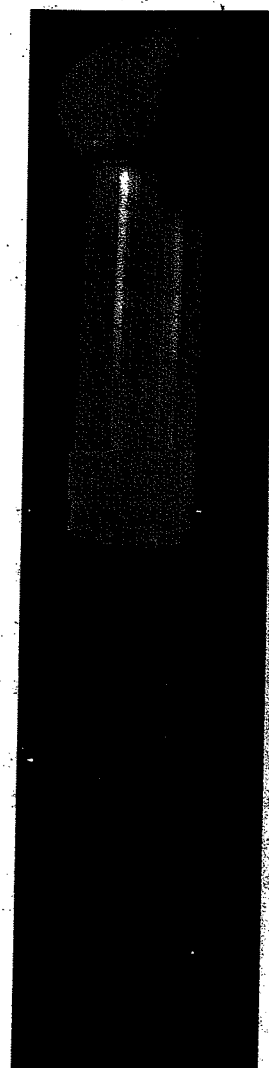


Fig. 8.7 Robertson's cooked meat medium.

9

Chapter

Identification of Bacteria

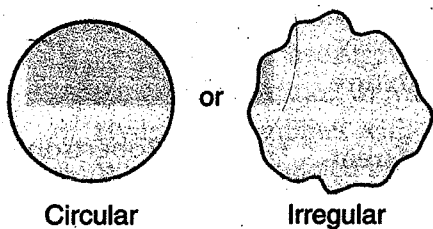
- List the important growth characters that are helpful in identifying bacteria cultured on solid medium, liquid medium, and on slope.

Growth characters helpful in identifying bacteria are:

Growth on Solid Medium (Colony Morphology)

The following characters of colony are noted:

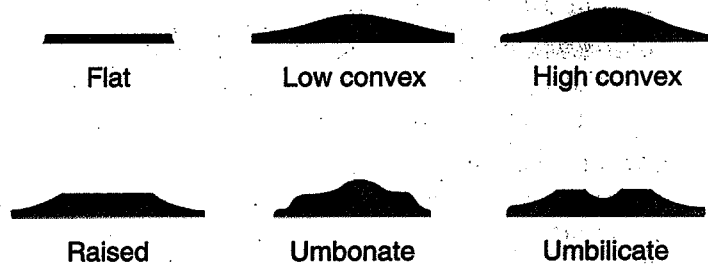
- Size—in millimeters
- Shape (Fig. 9.1)
- Surface—smooth, rough, granular
- Elevation (Fig. 9.2)
- Edge (Fig. 9.3)
- Opacity—opaque, translucent, transparent



Circular

Irregular

Fig. 9.1 Shape of colony.



Flat

Low convex

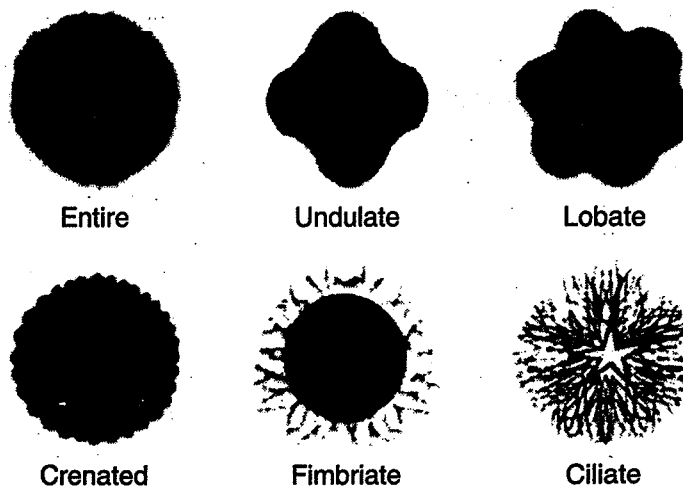
High convex

Raised

Umbonate

Umbilicate

Fig. 9.2 Elevation of colony.



Entire

Undulate

Lobate

Crenated

Fimbriate

Ciliate

Fig. 9.3 Edge of colony.

- Colour of colony
- Consistency—mucoid, friable, firm, butyrous
- Other properties
 - Haemolysis
 - Pigmentation
 - Swarming

Growth in Liquid Media (Broth Culture)

In a liquid medium (nutrient broth, peptone water or other liquid media), following characters are noted:

- The degree of growth—scanty, moderate or abundant
- Presence of turbidity and its nature (e.g. uniform turbidity)
- Presence of deposit, pellicle formation on surface and its quality

Growth on Slope Culture (Slope Reading)

In a slope culture, the following characters are noted:

- Degree of growth—scanty, moderate, abundant
- Surface—smooth, rough, granular
- Elevation—convex, flat, raised
- Edge—entire, undulate, crenated
- Opacity—opaque, translucent, transparent
- Consistency—firm, butyrous, powdery (dry), mucoid, membranous
- Colour of colonies—creamy white, yellow (golden or lemon yellow), bluish green, etc.
- Luster—glistening, dull
- Changes in medium, e.g. change in colour, pitting of agar, etc.

■ Mention the staining characters used in the identification of bacteria.

Study of staining characters of bacteria help in preliminary identification. Differential and special staining methods are used for this purpose. These include:

- *Gram staining*—helps to differentiate bacteria into Gram-positive and Gram-negative
- *Ziehl-Neelsen staining*—differentiates bacteria into acid-fast and nonacid-fast and helps in preliminary identification of mycobacteria from other bacteria
- Various *special staining methods* help to bring out special characters, e.g. Albert's stain is used to demonstrate metachromatic granules, which help in the identification of *Corynebacterium diphtheriae*—a causative agent of diphtheria

■ Mention the metabolic activities that assist in identification of bacteria.

Metabolic activities that help in classification of bacteria and differentiate species are:

- Requirements of oxygen
- Need for carbon dioxide
- Pigment production
- Power of haemolysis

■ Describe pointwise the various biochemical reactions used for identifying bacteria.

Biochemical reactions are used for accurate identification of bacteria. The most important and most widely used biochemical reactions are given below.

Sugar Fermentation

Principle

Used to determine the ability of a bacterium to ferment a specific sugar (carbohydrate) producing acid or acid with gas.

Fermentation medium (Fig. 9.4)

The important constituents are:

- Suitable basal medium—peptone water
- Sugar (1%)—glucose, lactose, sucrose and mannitol are routinely used
- Suitable indicator—Andrade's indicator (sodium hydroxide 1 acid fuchsin)
- Durham's tube—a small inverted tube filled with liquid for collection of gases
- The constituents are dispensed in test tubes and sterilized under low-pressure steam

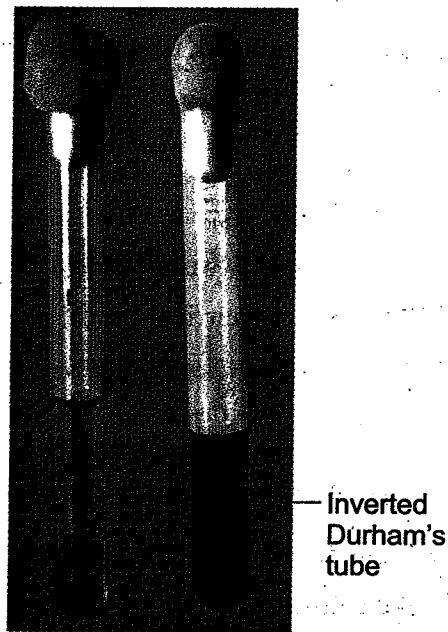


Fig. 9.4 Sugar fermentation.

Test procedure

A drop or a loopful of liquid culture or a suspension of solid culture in a sterile liquid is inoculated in a test medium and incubated for 18–24 hours at 37°C.

Result

The culture is observed for development of pinkish-red colour and gas production (positive test). No colour change of medium indicates negative test.

Mechanism

Fermentation of sugar results in the formation of acid and gas. The resultant acidity changes pH of the medium that is expressed as change in colour of the indicator from yellow to pinkish-red and the gas formed is collected in Durham's tube.

IMViC Tests

Indole Test (Fig. 9.5)

Principle

To demonstrate the ability of certain bacteria to decompose the amino acid tryptophan into indole.

Medium

Tryptophan rich medium—peptone water.

Test procedure

The test bacterium is inoculated into peptone water and incubated at 37°C for 24–48 hours. The production of indole is tested by adding indole reagent (Kovac's or Ehrlich's reagent). Xylene (1 ml) may be added before indole reagent.

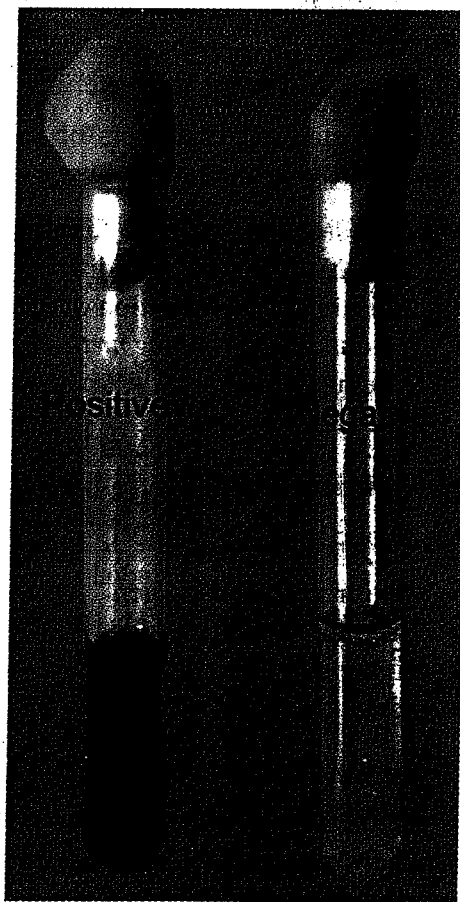


Fig. 9.5 Indole test.

Result

Development of red ring at the surface of the medium indicates positive test. Yellow coloured ring indicates negative test.

Mechanism

Tryptophan $\xrightarrow[\text{Kovac's reagent}]{\text{Bacterial decomposition}}$ Indole—red colour with

This indole is extracted by xylene and amyl alcohol present in Kovac's reagent in the surface layer, which reacts with para-dimethylaminobenzaldehyde and gives red colour.

Examples of positive and negative bacteria

- Indole-positive—*E. coli*, *Proteus vulgaris*
- Indole-negative—*Klebsiella* spp., *Proteus mirabilis*

Methyl Red Test (Fig. 9.6)**Principle**

To determine the ability of bacteria to produce considerable amount of acid from glucose.

Medium

Glucose phosphate broth (GPB).

Test procedure

The test bacterium is inoculated into GPB and incubated at 37°C for 24–28 hours. Acid production is tested by adding few drops of methyl red reagent.

Result

Development of bright red colour—positive test, yellow colour—negative test.

Mechanism

Some bacteria produce considerable amount of acid by fermenting glucose and resulting in a high concentration of hydrogen ions (that keeps pH below 4.5) that gives red colour when indicator is added.

Examples of positive and negative bacteria

- MR positive—*E. coli*, *Proteus* spp., *Salmonella* spp.
- MR negative—*Klebsiella*, *Enterobacter* spp.

Voges-Proskauer (VP) Test (Fig. 9.7)**Principle**

To determine the ability of bacteria to produce acetyl methyl carbinol (acetoin) from pyruvic acid.

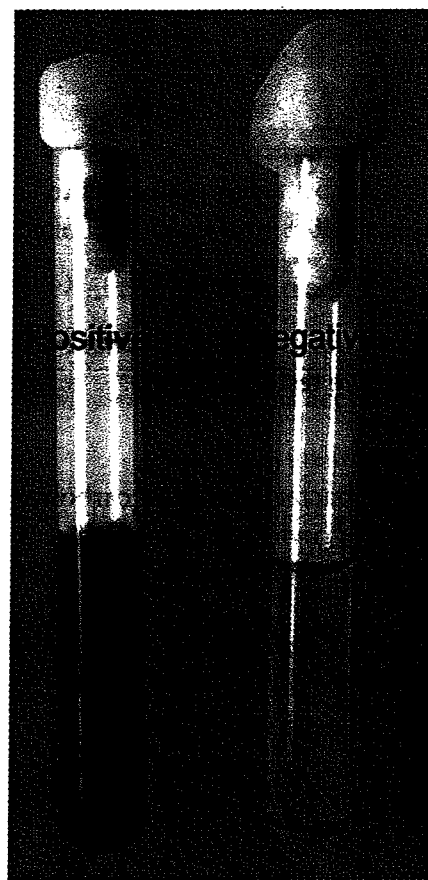


Fig. 9.6 Methyl red test.

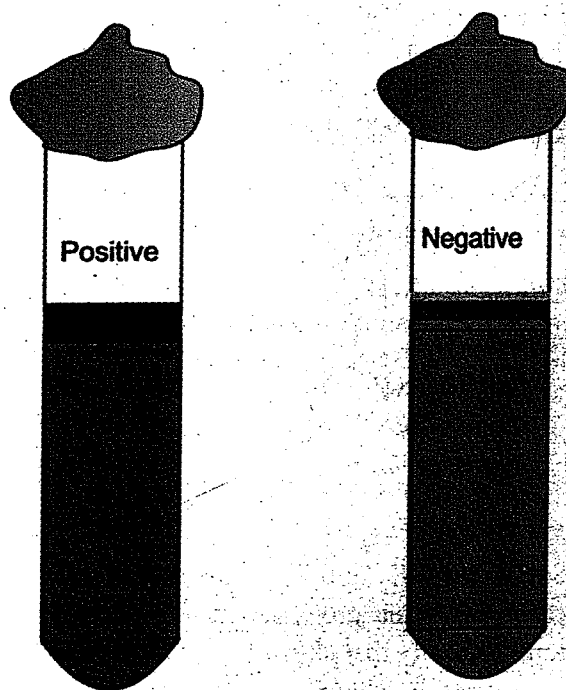


Fig. 9.7 Voges-Proskauer test.

Medium

GPB.

Test procedure

The test bacterium is inoculated into GPB and incubated at 37°C for 48 hours. After incubation, 1 ml of 40% KOH and 3 ml of 5% alpha-naphthol in absolute alcohol is added.

Result

Development of pink colour in 2–5 minutes—positive test, no pink colour in 30 minutes—negative test.

Mechanism

Some bacteria ferment carbohydrates with the production of acetyl methyl carbinol (acetoin) from pyruvic acid. In the presence of alkali (KOH) and atmospheric oxygen, acetoin is oxidized to diacetyl that gives pink colour.

Examples of positive and negative bacteria

- VP positive—*Klebsiella* spp., *Enterobacter*, El Tor *Vibrio*
- VP negative—*E. coli*, *Proteus* spp., *Salmonella* spp.

Citrate Utilization Test (Fig. 9.8)**Principle**

To determine the ability of bacteria to utilize citrate as the sole source of carbon for its growth.

Medium

Koser's citrate medium (liquid) or Simmons citrate medium (solid).

Test procedure

The test bacterium is inoculated into Koser's or Simmons citrate medium and incubated at 37°C for 24–96 hours.

Result

Koser's citrate medium

- Positive test—turbidity because of growth
- Negative test—no turbidity

Simmons citrate medium, this medium contains indicator—bromothymol blue

- Positive test—change of colour from green to blue and growth of bacterium
- Negative test—no colour change and no growth

Mechanism

Some bacteria utilize citrate as the sole source of carbon for their growth. Citrate is utilized with resultant alkalinity that alters the pH of the medium which consequently changes the colour of bromothymol blue from green to intense blue or makes the medium turbid in case of Koser's citrate medium.

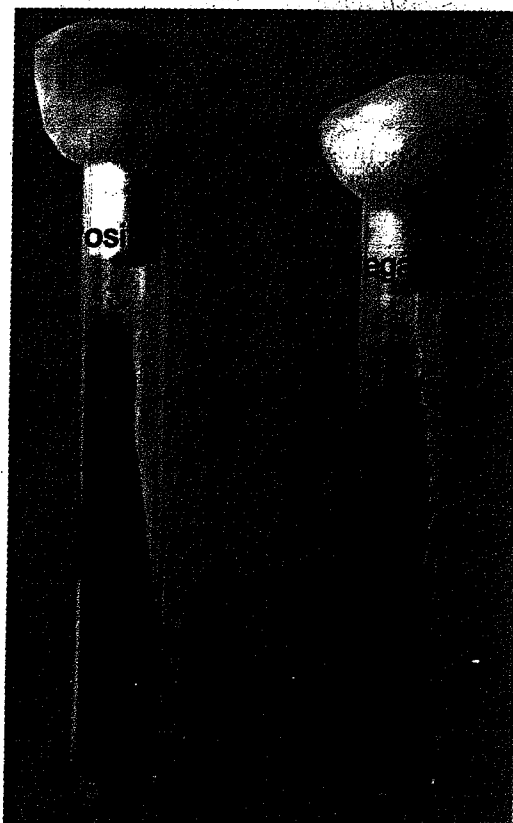


Fig. 9.8 Citrate utilization test.

Examples of positive and negative bacteria

- Citrate positive—*Klebsiella*, *Citrobacter*, *Pseudomonas aeruginosa*, *Enterobacter*, *Salmonella* except *Salmonella typhi*
- Citrate negative—*E. coli*, *S. typhi*, *Shigella*

Hydrogen Sulphide Production (H_2S) Test**Principle**

To determine the ability of bacteria to produce H_2S from sulphur-containing amino acids.

Medium

Peptone water or media containing lead acetate, ferric ammonium citrate or ferrous acetate.

Test procedure

Method I: Test bacterium is inoculated into liquid medium and a strip of lead acetate paper is held over inoculated culture in a test tube between the cotton plug and the tube. The strip is observed for blackening after overnight incubation.

Result

- Positive test—blackening or browning of lead acetate paper
- Negative test—no change in colour

Method II: Test bacterium is inoculated with a straight wire in a medium containing metallic salt (lead acetate, ferric ammonium citrate or ferrous acetate) and incubated for 7 days. The medium is observed daily.

Result

- Positive test—blackening of medium
- Negative test—no colour change

Mechanism

Some bacteria liberate H_2S by enzymatic action on sulphur-containing amino acids such as methionine, cysteine, and cysteine. The liberated H_2S causes blackening when it interacts with metallic salts.

Examples of positive and negative bacteria

- H_2S positive—*Proteus* and *Salmonella* spp. with some exceptions
- H_2S negative—*E. coli*, *Klebsiella*, *Salmonella paratyphi A*

Urease Test (Fig. 9.9)**Principle**

To determine the ability of bacteria to decompose urea into ammonia.

Medium

Christensen's urease agar.

Test procedure

The test bacterium is heavily inoculated on the entire slope surface and incubated at 37°C . The medium is observed after 4 hours and after overnight incubation.



Fig. 9.9 Urease test.

Result

- Positive test—development of pink colour
- Negative test—no change in colour

Mechanism

Some bacteria produce an enzyme urease that splits urea into ammonia. Ammonia makes the medium alkaline resulting in altered pH that causes a change in the colour of phenol red from pale yellow to pink.

Examples of positive and negative bacteria

- Urease positive—*Klebsiella*, *Proteus*, *Helicobacter pylori*
- Urease negative—*E. coli*, *Salmonella* spp.

Catalase Test**Test procedure**

Method I: One millilitre of hydrogen peroxide (H_2O_2) solution is poured over a 24 hours nutrient agar slope culture of the test bacterium and observed for gas bubbles immediately

Method II: A small amount of culture of test bacterium is picked from nutrient agar slope with the help of sterile glass rod and inserted into H_2O_2 solution and observed for gas bubbles

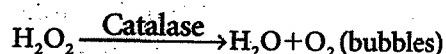
Method III: A drop of H_2O_2 is taken on a clean glass slide to which the small amount of culture of test bacterium is added with the help of glass rod and observed for gas bubbles

Result

- Positive test—immediate bubbling
- Negative test—no gas bubbles
- False positive—if culture containing catalase, e.g. from BA, or iron wire loop is used

Mechanism

Some bacteria produce an enzyme catalase that catalyses release of oxygen from H_2O_2



Hence, contact of bacteria with H_2O_2 causes formation of gas bubbles indicating formation of oxygen.

Examples of positive and negative bacteria

- Catalase positive—staphylococci, all members of Enterobacteriaceae except *Shigella dysenteriae* type I, *Bacillus* spp.
- Catalase negative—streptococci and pneumococci
- The test is primarily used for differentiation of staphylococci from streptococci

Oxidase Test**Principle**

To determine the ability of bacteria to produce an enzyme cytochrome oxidase.

Test procedure

Plate method: A freshly prepared 1% solution of tetra-methyl-para-phenylenediamine dihydrochloride (oxidase reagent) is poured on the surface of colonies of test bacterium and observed for development of purple colour. This test is useful to pickup the colonies of oxidase-positive bacteria from a mixed growth on culture media.

Dry filter paper method: A strip of filter paper soaked in a freshly prepared 1% solution of oxidase reagent is smeared with test bacterium and immediately observed for the deep purple colour.

Result

- Positive test—deep purple hue within 5–10 seconds
- Negative test—no colour

Mechanism

Some bacteria produce an enzyme cytochrome oxidase that catalyses the transport of electrons between electron donors in the bacteria and redox dye (oxidase reagent) which is reduced to deep purple colour.

Examples of positive and negative bacteria

- Oxidase positive—*Pseudomonas* spp., *Vibrio* spp., *Neisseria*, *Aeromonas*, *Alcaligenes*
- Oxidase negative—all members of Enterobacteriaceae, staphylococci, streptococci and many others (other than oxidase positive bacteria)

Coagulase Test (Fig. 9.10)

Principle

To determine the ability of *Staphylococcus aureus* to produce an enzyme coagulase that is responsible for clotting of human or rabbit plasma.

Test procedure

Slide method: Detects clumping factor (bound coagulase). This test is performed by mixing emulsified suspension of staphylococcal colonies with a drop of undiluted rabbit or human plasma and observed for prompt clumping which indicates positive test.

Result

- Positive test—prompt clumping
- Negative test—no clumping

Tube method: Detects real coagulase (free coagulase). This test is performed by mixing 0.1 ml of an overnight broth culture or an agar suspension of staphylococcal colonies with 0.5 ml of a 1:5 dilution of the rabbit or human plasma. It is observed for plasma clot after 3 to 6 hours incubation at 37°C in a water bath. EDTA (ethylenediaminetetraacetic acid), oxalate or heparin

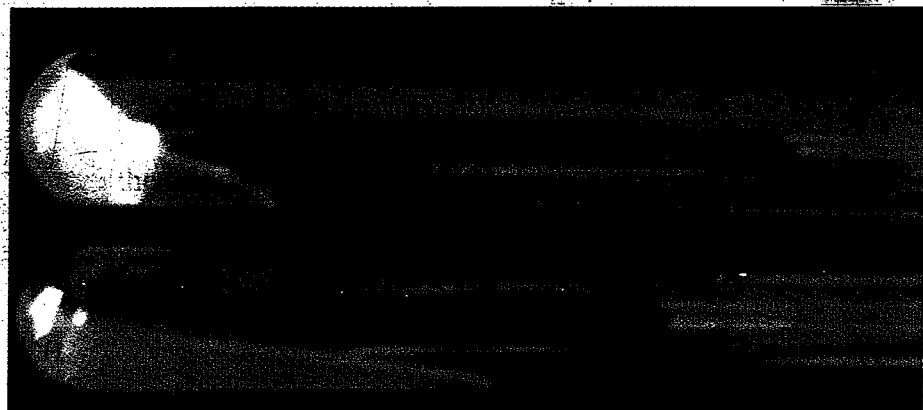


Fig. 9.10 Coagulase test.

may be used as anticoagulant for plasma preparation (citrate is not used because it may be utilized for some contaminant bacteria giving false positive results). This test is commonly used.

Result

- Positive test—formation of plasma clot that does not allow flowing of plasma when tube is inverted
- Negative test—no plasma clot

Mechanism

Staph. aureus produces an enzyme coagulase that acts along with a coagulase reacting factor present in human or rabbit plasma (absent in guinea pigs plasma, hence no clotting of guinea pigs plasma) and binds to prothrombin and convert fibrinogen to fibrin that results into clotting.

Examples of positive and negative bacteria

- Coagulase positive—*Staph. aureus*, *Yersinia pestis*
- Coagulase negative—*Staph. albus*, *Staph. citreus* and all other bacteria

Triple Sugar Iron (TSI) (Fig. 9.11)

Principle

To determine the ability of bacteria to ferment carbohydrates incorporated in a growth medium and production of H_2S .

Medium

TSI agar medium containing glucose, lactose, sucrose and ferric salts. Phenol red indicator is incorporated. The medium is used in the form of a butt and slant.

Test procedure

Test bacterium is inoculated deep in the butt with the help of straight wire (stab culture) and incubated at $37^\circ C$ overnight.

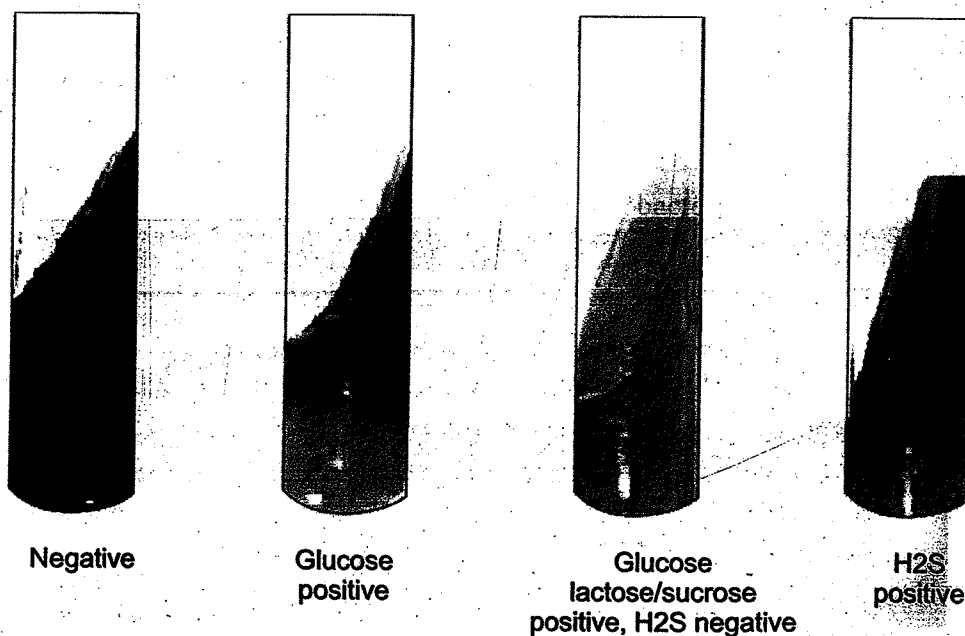


Fig. 9.11 Triple sugar iron test.

Result

If the slant remains red and the butt becomes yellow—all three sugars are fermented

- If bubbles are produced in the butt—indicates gas production
- Blackening of the medium—indicates H_2S production
- Red colour of the medium—indicates no fermentation

These biochemical reactions aid in the identification of most of the bacterial pathogens. In addition to these tests, some other biochemical reactions may be used to characterize them further or to identify some more complex bacteria. These additional tests used include:

- Phosphatase test
- DNase test
- Nitrate reduction test
- Phenylalanine deaminase pyruvic acid test (PPA)
- Growth in KCN—for identification of enteric bacteria
- Hippurate hydrolysis—for *Klebsiella*
- Egg yolk reaction—for *Cl. welchii*
- Gelatin liquefaction—for gelatin liquefying bacteria

■ **Mention the specific utility of following methods for identifying bacteria: antigenic analysis, bacteriophage and bacteriocin typing, pathogenicity tests and antibiotic sensitivity testing.**

The following are the identification methods and their utility under specific circumstances:

Antigenic Analysis

Antigenic analysis of test bacterium using specific antisera can be used for **identification** of bacterium or to **confirm further** the biochemically identified bacterium by agglutination or precipitation reaction.

Typing Methods

Methods such as bacteriophage typing and bacteriocin typing are used for intraspecies differentiation of some bacteria. These typing methods are useful in **epidemiological studies**.

Pathogenicity Tests

Pathogenicity tests are required sometimes to confirm the identity of pathogen, e.g. for **identification and establishing virulence** of *Corynebacterium diphtheriae*. Guinea pigs, rabbits and mice are commonly used. The animals may be injected by subcutaneous, intramuscular, intraperitoneal, intravenous, intracerebral, oral or nasal route, based upon the bacterium to be tested. The identification of bacterium is done based on the postmortem findings and other characters.

Antibiotic Sensitivity Testing

It not only helps in the **selection of antibiotics** of choice for treatment but also helps in identification of some bacteria, e.g. bacitracin sensitivity in identification of *Streptococcus pyogenes*, optochin sensitivity to identify pneumococci, etc.

10

Chapter

Sterilization and Disinfection

■ What do you understand by the terms sterilization and disinfection?

The terms sterilization and disinfection are generally used for the process of destruction or complete elimination of any living microorganism. The term sterilization is used for the physical methods and the term disinfection is used for the chemical methods.

■ Define sterilization. Describe in detail the various physical agents/methods of sterilization.

Sterilization is a process by which an article, surface or medium is made free of all microorganisms. It is the process of destruction or complete removal of all kinds of microorganisms, including spores.

Physical Methods of Sterilization

Important physical methods of sterilization include:

- Sunlight
- Drying
- Heat
 - Dry heat
 - Moist heat
- Radiation
 - Nonionizing radiation
 - Ionizing radiation
- Filtration

Sunlight

- It has germicidal activity due to ultraviolet rays present in it
- It is a natural method of sterilization that occurs spontaneously under natural conditions, e.g. sterilization of water in tanks, rivers and lakes

Drying

- Moisture is required for the growth of microbes
- Drying in air reduces the moisture content and produces deleterious effects
- Practically, it is unreliable and also spores are not affected

Heat

- It is the most reliable method of sterilization
- It is the method of choice for materials, which are not damaged by heat
- Two types of heat
 - Dry heat
 - Moist heat

Dry Heat

- Dry heat is preferred for sterilization of glassware such as glass syringes, test tubes, Petri plates and materials such as oils, jellies and powders
- It is unsuitable for materials such as fabrics, which may be damaged by dry heat
- It is a less efficient process than moist heat, as it requires longer time and higher temperature, e.g.
 - Bacterial spores may require a temperature of 140°C for 3 hours for killing.
 - Vegetative forms of bacteria may require a temperature of 100°C for 1 hour 30 minutes
- It has low penetration power, hence it is not effective when nonconducting materials protect microorganisms

Principle

Dry heat kills microorganisms by

1. Denaturation of their proteins
2. Oxidizing their chemical constituents (oxidative damage)
3. Toxic effects of elevated levels of electrolytes

Procedures for use of dry heat

Dry heat can be used in the following ways:

1. Incineration

- This is direct burning of materials at high temperature (800°C–1000°C)
- It is an efficient method of sterilization and disposal of contaminated materials by direct burning
- Used for destroying materials such as
 - Soiled dressings
 - Beddings of patient
 - Animal carcasses
 - Pathological materials

2. Red heat

- It is direct heating of an instrument or an object in a flame till it becomes red hot
- It is an easy way of sterilization but has limited applications
- Used for sterilizing noninflammable materials such as
 - Inoculating loops or wires
 - Tips of forceps
 - Scissors
 - Spatulas

3. Flaming

- Direct exposure for a few seconds by passing a few times through the Bunsen flame without allowing them to become red-hot
- May be used for
 - Scalpels
 - Needles
 - Glass slides
 - Cover slips
 - Mouth of culture tubes
 - Neck of flasks, etc.
- Its efficacy is not certain

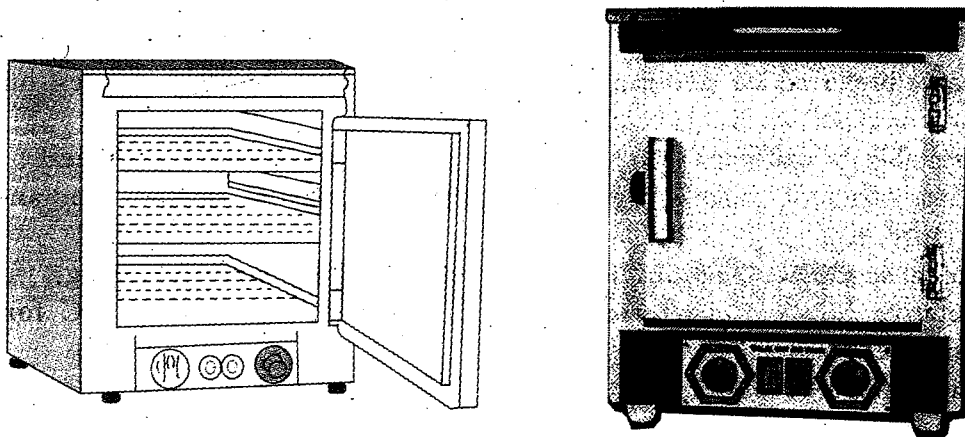


Fig. 10.1 Hot air oven.

4. Hot air sterilizers (ovens) (Fig. 10.1)

- Hot air oven is the most widely used method of sterilization by dry heat
- It has a two-walled chamber
 - Inner wall of copper vessel
 - Outer wall of asbestos to reduce radiation of heat
- The chamber is divided into small compartments with the help of removable racks
- Thermometer is inserted at one of the corners
- It is heated by electricity, with heating elements in the wall of the chamber
- A fan is also fitted to ensure even distribution of air and elimination of air pockets

Directions for use

- It is operated at 160°C for one hour or at 180°C for 30 minutes
- All glassware to be sterilized are covered with craft paper and kept in a chamber in a manner, which allows free circulation of air in between the objects
- The temperature is adjusted at the required level
- The temperature is recorded with the help of thermometer and maintained for one hour at 160°C or 30 minutes at 180°C
- After this specific time, electricity is cut off and the material is allowed to cool
- On cooling, the material is taken out from oven and used

Uses

- Used for sterilization of glassware such as flasks, pipettes, test tubes, Petri plates, etc.
- Used for sterilization of forceps, scissors, scalpels and other surgical instruments
- For sterilization of swabs
- For sterilization of pharmaceutical products such as liquid paraffin, sulphonamides, dusting powder, fats, grease, etc.
- For cutting instruments, e.g. those used in ophthalmic surgery—kept for 2 hours at 150°C

Precautions

- Oven should not be overloaded
- Glassware must be free from water traces and properly covered with craft paper
- The material should be properly arranged to allow free circulation of air
- Rubber material except for silicon rubber or any inflammable material should not be kept inside the oven
- It should be allowed to cool for 2 hours before opening the door to avoid cracking of glassware by sudden cooling

Sterilization control

To ensure the proper sterilization, various controls are used during sterilization. These include:

- The paper strips impregnated with spores (10^6) of nontoxic strain of *Clostridium tetani* or *Bacillus subtilis* are placed along with material to be sterilized. After sterilization, the strips are transferred to suitable media and incubated for appropriate time at 37°C . The medium is observed for growth, if it shows growth then process of sterilization is considered as faulty and if no growth is seen then the sterilization is considered as perfect
- Browne's tube is convenient for routine use. A tube placed with material shows change in colour from red to green depending upon the temperature. Green spot indicates that the process of sterilization is perfect
- Thermocouples may also be inserted with the material and temperature can be recorded

Moist Heat

- It is more efficient than dry heat in penetrating the material
- It is commonly used for the sterilization of culture media prior to washing, laboratory coat, apron, glass syringes, surgical instruments, etc.
- Moist heat may be employed in the following ways:
 - Temperature below 100°C
 - Temperature around 100°C
 - Temperature above 100°C

1. Temperature below 100°C **A. Pasteurization**

- Used for sterilization of milk
- Two methods:
 - Holder method of pasteurization (63°C for 30 minutes) and
 - Flash method of pasteurization (72°C for 15–20 seconds followed by quick cooling to 13°C or lower)
- All nonsporing pathogens are killed except *Coxiella burnetii*, which is relatively resistant and may survive the holder method
- Spores may not be destroyed

B. Inspissation

For sterilization of media such as Lowenstein–Jensen's and Loeffler's serum slope.

Method: Heating at $80\text{--}85^\circ\text{C}$ for 30 minutes on three successive days in inspissator.

Principle

- First exposure on day 1st kills all vegetative forms
- Spores, which are not destroyed, would germinate to form vegetative forms before the second exposure
- Second exposure on day 2nd kills newly formed vegetative forms
- Spores, if at all present, germinate till third exposure and would be killed on third exposure on day 3rd ensuring complete sterilization

C. Water bath

For sterilization of serum or body fluids containing coagulable protein.

Method: Heating for 1 hour at 56°C on several successive days in water bath.

D. Vaccine bath

For inactivation of nonsporing bacteria for preparation of vaccines.

Method: Heating for 1 hour at 60°C in a vaccine bath.

2. Temperature around 100°C

A. Boiling

- Boiling at 100°C for 10–30 minutes kills almost all vegetative bacteria but does not ensure complete sterilization, as some spore forming bacteria may not be eliminated completely, e.g. *Cl. tetani* may survive boiling of 1–3 hours
- Addition of 2% sodium carbonate promotes sterilization
- Recommended for sterilization of pipettes, cylinders, rubber stoppers, scalpels, forceps, scissors, syringes, etc. when absolute sterility is not required
- Not recommended for sterilization of instruments used in surgical procedures

B. Steaming

- Steaming at atmospheric pressure at 100°C is used for sterilization of culture media, e.g. media containing sugars and gelatin, which may be damaged at higher temperature
- **Koch or Arnold steam sterilizer** is used for this purpose
- One exposure for 90 minutes ensures complete sterilization

C. Tyndallization

- It is an intermittent or fractional sterilization method
- In this, steaming of object is done at 100°C for 30 minutes on three successive days
- This ensures complete removal of spores, which might not be killed by a single steaming

Principle

First exposure kills vegetative forms. The spores present will germinate on successive days and will be killed by second and third exposure.

This method can be applied only to nutrient media in which spores can germinate in the intervals between steaming.

3. Temperature above 100°C

- It is the most widely used method of sterilization
- Sterilization by steam under pressure is carried out at temperature between 108°C and 147°C
- By using appropriate temperature and time, a variety of materials can be sterilized
- Several types of steam sterilizers are available. The commonest one is *autoclave*

Autoclave (Fig. 10.2)

- Water boils when its vapour pressure equals that of the surrounding atmosphere
- Water boils at 100°C when its vapour pressure is same as that of atmospheric pressure but when the pressure inside a closed vessel increases, the temperature at which water boils also increases (Table 10.1)
- Saturated steam (steam above 100°C) has a better penetrating power and microorganisms are more susceptible to moist heat
- When steam comes in contact with a cooler surface it condenses to water and liberates its latent heat to that surface, e.g. 1600 ml of steam at 100°C and at atmospheric pressure condenses into 1 ml of water at 100°C and liberates 518 calories of heat
- The large reduction in volume sucks in more steam to the same site and the process continues till the temperature of that surface is raised to that of the steam
- The condensed water produces moist conditions for killing the microbes present

Structure of autoclave

- It consists a vertical or a horizontal cylinder of gunmetal or stainless steel, supported by a case made of iron sheet

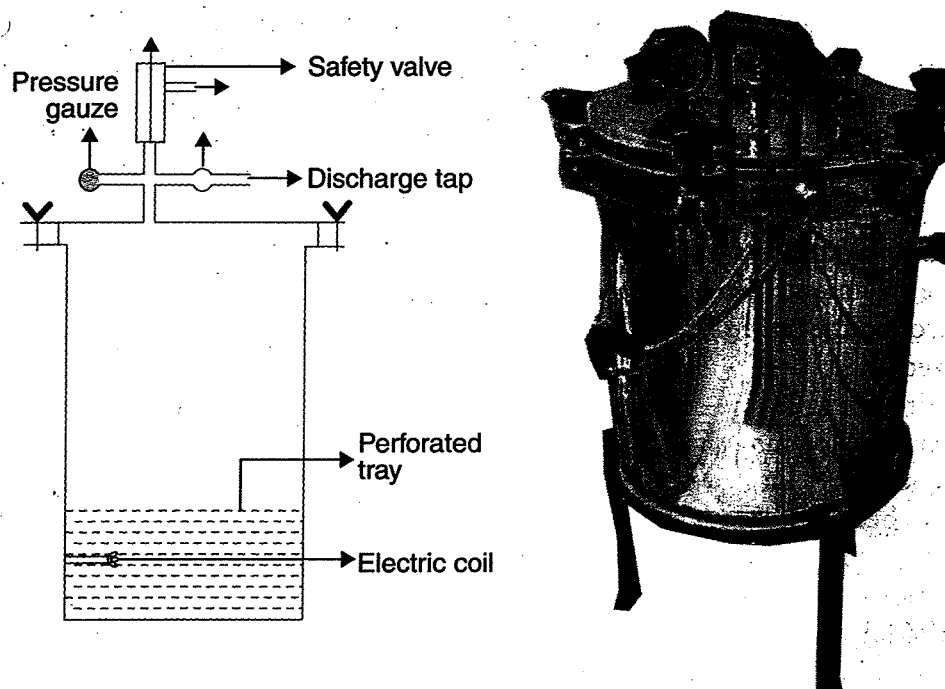


Fig. 10.2 Autoclave.

Table 10.1 Relationship between pressure and temperature

Pressure	Temperature
Atmospheric pressure	100°C
5 lb/inch ²	109°C
10 lb/inch ²	115°C
15 lb/inch ²	121°C
20 lb/inch ²	126°C
25 lb/inch ²	130°C
30 lb/inch ²	135°C

- The lid or door is heavy, secured by butterfly nuts and rendered airtight by an asbestos washer
- The lid has three screws – Steam outlet, safety valve and pressure indicator
- The autoclave is heated by a gas burner or electricity

Directions for use

- Sufficient water is placed in cylinder
- Articles to be sterilized are placed on a platform (tray)
- The lid is closed properly and screws are tightened
- The safety valve is adjusted to the required pressure
- Heating is started with steam tap kept open to displace air inside
- When all the air inside is expelled out (indicated by continuous flow of steam coming out of the tap after some time), the tap is closed
- The pressure is recorded; the pressure usually employed is 15 lb (7.5 kg) per square inch above the atmospheric pressure, at this pressure water boils at 121°C
- This pressure is maintained for 15 minutes, which ensures killing of all microorganisms including the spores

- After the desired holding period, the heater is turned off and autoclave is allowed to cool till the pressure equals the atmospheric pressure (till indicator bar comes to a null point)
- Steam tap is opened slowly to allow the air to enter inside
- The lid is opened and material is taken out for use

Precautions

- All air must be expelled out of the autoclave. The presence of air lowers down the temperature of the steam and affects the process of sterilization. Also, the presence of air forms air pockets around the material and thereby prevents penetration of the steam in the material to be sterilized
- To avoid drenching by the condensed steam, the cotton wool plugs should be covered with craft paper or cellophane sheets
- The lid should be opened when the outside and the inside pressure is equal. This precaution is necessary because opening of lid when the pressure inside is high will cause violent boiling of the medium/liquid at a temperature above 100°C and may sometimes be expelled from the container with an explosive force
- Opening of lid when the pressure inside is low, i.e. below atmospheric pressure, will cause evaporation of water and loss of water from the medium/liquid reducing the quantity of the medium/liquid

Uses

The autoclaving can be used for sterilizing anything which is not damaged by steam. These include:

1. All solid and liquid media
2. Distilled water
3. Saline solution
4. Laboratory coats
5. Swabs, syringes and needles
6. Surgical instruments
7. Dressing material
8. Laboratory ware
9. Pharmaceutical products

Sterilization control

Sterilization of various objects/articles is of prime importance for various purposes. So to confirm whether the objects/articles are properly sterilized or not, various controls are introduced during sterilization. For determining the efficacy of autoclave by

1. **Biological control:** The paper strips impregnated with *Bacillus subtilis* or paper strips or ampoules containing spores of *Bacillus stearothermophilus* are introduced with material to be sterilized. After sterilization, the strips or ampoules are transferred to suitable media and incubated for an appropriate time at 37°C. The medium is observed for growth, if it shows growth, the sterilization is considered as imperfect and if it shows no growth then it is considered as perfect
2. **Physical control:**
 - Chemical dyes showing change in colour at specific temperature
 - Chemicals of known melting point. Melting at a particular temperature indicates whether the temperature has reached the desired level or not
 - Use of thermocouples
 - Use of autoclave tapes

Radiation

- The radiation has effect on microorganisms because they absorb radiant energy
- The time required for destruction of microorganisms depends upon:
 - Intensity of radiation
 - Duration
 - Distance of the source of illumination
 - Nature of medium in which the organisms are exposed
- Types—two types of radiations are used for sterilization
 - Nonionizing radiation
 - Ionizing radiation

1. Nonionizing Radiation

Ultraviolet radiations (UV rays)

- These are short rays (wavelength 210–310 nm) having low penetrating power
- Effective wavelength is between 240 nm and 280 nm; **254 nm** is the most efficient wavelength for sterilization
- These are mutagenic and produce lethal photochemical changes in enzymes and cell constituents such as DNA, protein, etc.
- UV rays are able to kill cells, and temporarily delay cell division and synthesis of certain substances by the cell
- Most effective in absence of oxygen
- All living microorganisms (bacteria, fungi, viruses) including spores are sensitive to UV treatment. However, spores require double exposure

Applications

- In the control of air borne infections by air disinfection, e.g. disinfection of enclosed areas such as
 - Entry ways
 - Hospital wards
 - Operating rooms
 - Laboratories
- Disinfection of drinking water and to obtain pyrogen-free water
- Preparation of bacterial and viral vaccines

Sources

- UV rays are components of sunlight, because of which sunlight has got bactericidal activity
- Special UV lamps – the most commonly employed one is the low-pressure mercury vapour type, which emits more than 95% radiation of wavelength 254 nm

2. Ionizing Radiation

- These include gamma rays, beta rays, X-rays, etc.
- They have very high penetrating power
- These are highly lethal to DNA and other vital cell constituents
- The method is known as **cold sterilization** as there is no appreciable increase in temperature
- Gamma and beta rays are extensively used for sterilization of instruments and dressing packs such as
 - Plastic syringes
 - Swabs
 - Culture plates

- Catheters
- Various type of rubbers
- Cardboards
- Fabrics
- Metal foils
- Also for sterilization of oils and greases, food products, pharmaceutical products, etc.
- X-rays and other type of ionizing radiations (alpha particles, neutrons, protons) are not recommended for sterilization either because of poor penetration power or because they induce radioactivity in exposed materials

Filtration

- Method of sterilization for **thermolabile substances**
- Filters are used for removal of microorganisms from the fluids or liquids that are thermolabile (sensitive to heat, hence cannot be sterilized by heat)
- These thermolabile materials are made free from organisms by passing through filters
- Filtration is usually carried out under negative pressure
- The fluid is sucked through the filter in a receiving flask, which is connected to an exhaust pump
- The exhaust pump causes suction of the fluid through the filter

Applications

- Sterilization of medicines or other materials, which are heat labile and are damaged by heating, e.g.
 - Antibiotic solutions
 - Sera
 - Carbohydrate solutions
 - Enzyme solutions
 - Toxins or toxoids
 - Hydatid fluid
- To obtain cell-free bacterial products such as toxins, enzymes from cultures
- To make virus (bacteriophages) containing fluids free from bacteria, i.e. to obtain bacteria-free filtrates of viruses
- To separate and study microorganisms, which are scanty in fluids, e.g. testing of water sample for *V. cholerae* and *Salmonella*
- For purification of water

Types of Filters

Different types of filters, each one having its own applications, are available.

Candle filters

- These are manufactured in different grades of porosity, in the form of hollow candles
- Used for purification of water on large scale
- Two types of candle filters are available:
 1. **Unglazed ceramic filters** also called **porcelain filter** composed of hydrous aluminium silicate or kaolin, e.g. Chamberland and Doulton filters
 - After use they can be cleaned with chemical agents such as sodium hypochlorite
 - They withstand scrubbing
 2. **Diatomaceous earth filters**
 - These are composed of diatomaceous earth, asbestos and plaster of Paris in different proportions

- After use they can be cleaned with hypochlorite solution but they do not withstand scrubbing, e.g. Berkefeld filter

Asbestos filters

- Composed of asbestos fibres (magnesium silicate)
- Disposable, single-use discs, available in different grades, e.g. Seitz filter, Carlson and Sterimat filter

Disadvantages

1. Alkalinize filtered liquids
2. Has carcinogenic potential

Sintered glass filters

- Composed of finely powdered glass particles of different sizes according to the required pore size
- They have low absorptive property
- They can be cleaned easily
- They are expensive and being glass, fragile

Membrane filters

- Composed of cellulose esters and are called **Millipore** or **polypore filter**
- Available in the different range of pore size (0.05–12 μ); 0.22 μ filter is commonly used as this size is smaller than bacteria
- These are most suitable for preparing sterile solutions
- Used for
 - Water purification and analysis
 - Sterilization and sterility testing
 - Preparation of solution for parenteral use

■ Define disinfection. Name and in one sentence mention the mode of action of the chemical agents used for disinfection.

Disinfection is a process of destroying infectious agents. It differs from sterilization, as it does not kill the bacterial spores.

- **Disinfectant:** An agent, which is usually a chemical that kills the growing organisms. It may not affect bacterial spores
- **Antiseptic:** An agent that kills or inhibits the growth of microorganisms in contact with the body without causing extensive damage to the body tissue. These agents can be safely applied to living tissue
- **Germicide:** An agent capable of killing microbes
- **Bacteriostatic:** An agent capable of inhibiting growth of bacteria
- **Bactericide:** An agent capable of killing bacteria
- **Fungistatic:** An agent capable of inhibiting growth of fungi
- **Fungicide:** An agent capable of killing fungi

■ List the characteristics of an ideal disinfectant.

An ideal disinfectant must

- Have wide spectrum of antimicrobial activity and effective against vegetative and spore forming microorganisms
- Be stable—should not undergo any chemical changes
- Act in the presence of organic matters

- Be soluble in water or other suitable solvent
- Be effective in low concentration
- Be cheap and easily available
- Be odourless or have pleasant odour
- Not corrode or stain material to be disinfected
- Be effective in acid as well as alkaline medium
- Have speedy action and high penetrating power
- Be safe and easy to use—free from adverse effects and not cause local irritation
- Not be toxic, if absorbed into circulation

■ Describe briefly the commonly used disinfectants.

The commonly used disinfectants are:

1. Phenols

- Phenolic compounds are most widely used disinfectants
- Phenols are bactericidal or bacteriostatic in nature depending upon the concentration used. Some of them are fungicidal also
- Different phenolic compounds are:

A. Phenol or Carbolic Acid

- Phenol 1% solution has bactericidal activity, i.e. it damages the cell membrane and causes lysis
- Activity—it is effective against
 - Vegetative forms of bacteria
 - *Mycobacterium tuberculosis*
 - Certain fungi
- Excellent disinfectant for faeces, blood, pus, sputum, etc.
- Can also be used for disinfecting
 - Contaminated instruments and utensils
 - Contaminated linen and clothes (5%)
 - Carbonization of theatre (5%)

B. Cresol

- Because of low solubility in water, phenols are formulated with soaps, this enhances the bactericidal activity of phenols, e.g. Lysol is a solution of cresol in soap
- Cresols are more germicidal and less poisonous than phenols, but they are corrosive to living tissue, hence prolonged contact should be avoided

Uses

1. For cleaning floors (1% solution)
2. For disinfection of surgical instruments
3. For disinfection of glassware
4. For disinfection of excreta
5. For disinfection of furniture, tables and other contaminated objects

C. Halogenated Diphenyl Compounds

Examples

- Hexachlorophene—toxic, should be used with caution
- Chlorohexidine—highly effective against Gram-positive bacteria and also against Gram-negative bacteria. Less toxic/nontoxic. Used as a skin antiseptic, bladder irrigant. Incorporated in cosmetics and soaps. Used in the treatment of wounds.

2. Alcohols

- Ethyl alcohol (ethanol) and isopropyl alcohol are used as skin antiseptics
- Act by denaturing bacterial proteins. Also by disorganizing the lipid structure of the cell membrane
- No action against spores and viruses
- Effective at a concentration of 60–70% in water
- Isopropyl alcohol is a better fat solvent, more bactericidal and less volatile

Uses

1. Isopropyl alcohol is used for disinfection of clinical thermometer and ethanol is used to disinfect skin prior to cutaneous injections
2. Methyl alcohol is effective against fungal spores and used for disinfection of cabinets and incubators infected by fungi. It is less effective against bacteria and injurious to eyes when exposed to its vapours

3. Oxidizing Agents

- This group includes halogens, hydrogen peroxide, potassium permanganate, sodium perborate, etc.
- They are good disinfectants/antiseptics, but are less effective in presence of organic matter

A. Halogens

- i. Chlorine
- ii. Iodine

Chlorine

Chlorine and its compounds are used for

- Disinfection of water—water purification
- Disinfection of dairy equipments
- Disinfection of sanitary utensils
- In food industry
- For air disinfection (sodium hypochlorite in the form of aerosol) is most commonly used. Inorganic and organic chloramines are the chlorine compounds used for disinfection. This is due to release of free chlorine that reacts with water to form hypochlorous acid, which is a strong oxidizing agent that makes it an effective disinfectant
- Organic chloramines—used as antiseptic for wound dressing

Iodine

- Commonly used as a skin disinfectant
- Bactericidal in nature with moderate action against spores; also active against tubercle bacilli and many viruses
- Usually employed as a tincture—an alcoholic solution containing iodine (2.5%) and potassium iodide (2.5%) in alcohol (90%)
- Iodine combined with surface-active agents known as iodophores—are generally used and are claimed to be more active than tincture iodine

B. Hydrogen Peroxide (H_2O_2)

- It is a weak disinfectant used in a concentration of 3% solution.
- Useful for cleaning wounds, and for mouthwash or gargle

C. Potassium Permanganate

- It is a powerful oxidizing agent used in the treatment of urethritis
- Bactericidal in nature, also active against viruses

4. Acids and Alkalis

- Acids such as sulphuric acid, nitric acid, hydrochloric acid, benzoic acid, etc. and alkalis like potassium and sodium hydroxide and ammonium hydroxide are germicidal in nature
- They kill microorganisms by hydrolysis and altering pH of the medium
- They are rarely used as disinfectants
- Benzoic acid and acetic acid are used as preservatives

5. Dyes

- Various dyes used for staining bacteria are bacteriostatic in nature
- Crystal violet, malachite green, brilliant green, etc. are active against Gram-positive organisms but have less effect on Gram-negative organisms
- They are used as skin and wound antiseptics

6. Heavy Metals

- Soluble salts of mercury, silver, copper, arsenic and other heavy metals have antibacterial activity—bactericidal and bacteriostatic activity
- Mercury chloride is used as disinfectant
- Organic compounds like merthiolate and mercurochrome are less toxic and useful as antiseptics
- Silver compounds—are widely used as antiseptics
- Organic silver salts—are efficient bactericidal agents
- Silver nitrate is bactericidal for gonococci and is used as a prophylactic agent in ophthalmia neonatorum in newborn infants
- Arsenic compounds were used in the treatment of syphilis in the past before the discovery of penicillin

7. Alkylating Agents

- Formaldehyde, glutaraldehyde and ethylene oxide are the alkylating agents
- They exert lethal effect on proteins

A. Formaldehyde

- In aqueous solution, it is bactericidal, sporocidal and also effective against viruses
- Formalin, an aqueous solution of 37% formaldehyde, is used for coagulating and preserving fresh tissues (specimens) and for destroying anthrax spores in hair and wool
- The gas is used for
 - fumigation of operation theatre, wards, sick rooms, laboratories
 - sterilization of instruments and heat sensitive catheters
 - clothing, bedding, furniture, books, etc.
- For fumigation of 1000 cu. ft. or 28.3 cu. meter room 150 g of potassium permanganate is added to 280 ml of formalin after sealing the windows and other outlets to generate gas
- Once the generation of formaldehyde vapours is started, the door should be sealed and left closed for 48 hours
- After disinfection, all traces of vapour should be nullified by exposure to ammonia vapour to avoid irritation and toxic effects

B. Ethylene Oxide

- It is an alkylating agent used for gaseous sterilization
- It is a colourless liquid with a boiling point of 10.7°C
- It is active against all kinds of bacteria, spores and viruses
- Act by inhibiting proteins and nucleic acids
- It is highly inflammable, mutagenic and carcinogenic
- It can be used for sterilization of any object but it is especially useful to sterilize those objects which are damaged by heat, e.g. heart-lung machine, respirators, sutures, dental equipments, etc.

C. Glutaraldehyde

- It has an action similar to formaldehyde
- It is especially effective against tubercle bacilli, fungi and viruses
- It is less toxic and irritant to eyes and skin than formaldehyde
- It can be used as a buffered solution for cleaning cystoscopes and bronchoscopes, corrugated rubber anaesthetic tubes and face masks, plastic endotracheal tubes, metal instruments and polythene tubing

8. Betapropiolactone (BPL)

- It is a condensation product of ketane and formaldehyde with a boiling point of 163°C
- It has a low penetrating power but it is more efficient than formaldehyde for fumigation purpose
- Active against all microorganisms and more active against viruses
- 0.2% BPL is used for sterilization of biological products
- It has carcinogenic activity

9. Surface Active Agents

- These agents alter energy relationship at interfaces, producing a reduction of surface or interfacial tension
- They are widely used as detergents, wetting agents and emulsifiers
- They are of the following four types:

A. Cationic Surface-active Agents

- These include quaternary ammonium compounds, which are more active at alkaline pH, e.g. cetyltrimethylammonium bromide (cetavlon or cetrinide) and benzalkonium chloride
- They act by denaturing proteins
- These are bactericidal in nature, more active against Gram-positive bacteria and to a lesser extent against Gram-negative bacteria
- No activity against spores, tubercle bacilli and most viruses

B. Anionic Surface-active Agents

- These include soaps, which act better at acidic pH
- They are prepared by using saturated and unsaturated fatty acids
- The soaps prepared from saturated fatty acids, e.g. coconut oil, are more effective against Gram-negative organisms
- The soaps prepared from unsaturated fatty acids, e.g. oleic acid, are more active against Gram-positive bacilli and *Neisseria*

C. Nonionic Surface-active Agents

These are relatively nontoxic and some of them may promote the growth of bacteria, e.g. Tween 80 promotes growth of tubercle bacilli.

D. Amphoteric or Ampholytic Compounds

These are active against a wide range of Gram-positive and Gram-negative bacteria and some viruses. These are known as 'Tego' compounds and are not in general use.

■ Write short notes on the following methods of determining the quality of disinfectants: (a) Rideal-Walker test, (b) Chick-Martin test, (c) In-Use test.

Rideal-Walker Test

In this test, similar quantities of organisms are subjected to varying concentration of phenol and disinfectant to be tested. The dilution of the test disinfectant, which sterilizes the suspension in a given time, is divided by the dilution of phenol, which sterilizes the suspension at the same time to obtain phenol coefficient. This test has many limitations and gives no idea about how the test disinfectant will function in presence of organic matter.

Chick-Martin Test

It is modification of Rideal-Walker test. In this, the test disinfectant is allowed to act in the presence of organic matter (3% dried human faeces or dried yeast) to simulate natural conditions.

This modification also has limitations, hence Chick-Martin test, as also Rideal-Walker test is no longer in use.

In-Use Test

In this, the efficacy of the disinfectant used in the hospital is assessed quantitatively. The efficacy is determined by its ability to inactivate a known number of a standard pathogenic *Staph. aureus* on a given surface within a certain time. Based on the results the dilution of disinfectant at which it is most effective is determined.

11

Chapter

Hospital Waste Management

■ What is Hospital waste? How would you classify hospital waste for management purposes?

- Hospital waste refers to all waste—biological or nonbiological—that is discarded and is not intended for further use in the hospital
- Two classes of hospital waste are recognized, these are:

Infectious Waste

A waste generated in hospital, which is capable of causing infectious disease or disability. It includes microbiological waste, blood and body fluids, contaminated laboratory wastes, sharps, pathological waste, soiled dressing, cotton, plaster and other material contaminated with blood and body fluids, bedding, cotton swabs, animal wastes generated by animal experiments, etc.

Noninfectious Waste

- A waste generated in hospital, which is not infectious and does not cause any hazard is known as noninfectious waste.

The amount of waste generated per bed per day varies with the type of hospital. However, on an average 1–5 kg of waste per bed per day is generated. The type of waste generated is 80–85% noninfectious, 10–15% infectious and 5% chemical waste, which is noninfectious but hazardous. These figures show that out of the total waste generated, only a small fraction of waste is infectious or hazardous.

■ What is the chief objective of hospital waste management? Highlight the health hazards associated with improper waste management.

The main aim of hospital waste management is:

- To prevent injury and accidental transmission of microbial infections to hospital staff, patients, attendants of patients, visitors and general public.
- Hence, proper hospital waste management programme for disposal of waste is of utmost importance for the safety of health care personnel as well as the community. Now, it is mandatory for every hospital to have proper waste management programme for its disposal.
- **Health hazards** associated with poor/improper hospital waste management programme are:
 - Higher rates of nosocomial infections
 - Injuries from sharps to health care personnel and waste handlers

■ Why is it important to segregate hospital waste before disposing it off? Highlight the benefits of good segregation practices. How is segregation of hospital waste managed according to Biomedical Waste Rules 1998?

Importance of Segregating Hospital Waste

Generation of waste (infectious and noninfectious) is a continuous process, hence proper collection and segregation is essential.

As 80–85% of waste generated in the hospital is noninfectious waste, it is crucial to prevent the mixing of infectious and noninfectious wastes. This can be accomplished if effective separation is done at the source, i.e. at the point of generation. It is the most important step to safeguard the occupational health of health care personnel and also it does not pose a significant health hazard to the general public. Thus, the effective segregation is the golden key to good management plan.

Benefits of Good Segregation Practices

- Minimize biomedical waste
- Reduce costs/expenditure
- Reduce impact of hazards to health care workers/community

Management of Segregated Waste According to Biomedical Waste Rules (1998)

Segregation of waste is done in different colour coded plastic (polythene) bags and containers (Biomedical Waste [BMW] Rules 1998) as different types of wastes need different type of treatment for their disposal. The recommended segregation and colour coding is as follows:

- **Yellow** plastic bag and yellow container—for human anatomical waste—all removed parts of the body during operation
- **Red** plastic bag and red container—for infectious waste such as cotton, gauze, bandages, plasters, etc. which are soaked/in contact with blood, urine, pus, or other body fluids
- **Blue** plastic bag and blue container—for plastic and rubber disposables such as IV sets, syringes, catheters, gloves, rubber tubes, etc.
- **Transparent** puncture proof container (white/blue)—for sharps such as surgical destroyed needles, blades, lancets, cut ampoules, glass vials, butterfly needles, suturing needles, etc.

All the above containers should have biohazard symbol.

■ Write an account of the measures taken for treatment and/or disposal of the already segregated hospital waste.

- Yellow bag containing anatomical waste does not need any treatment in hospital. It is directly sent for disposal—deep burial or incineration
- Red bag containing infectious waste can also be sent without any treatment for disposal—deep burial or incineration. If it contains highly infectious material, it should be sterilized immediately by autoclaving and then sent for disposal—by land filling
- Blue bag containing plastic and rubber disposables need treatment before disposal. These items are to be mutilated—cut into pieces to avoid reuse and disinfected in the ward by putting them into 1% bleaching solution for 30 minutes or in sodium hypochlorite solution and autoclaved. Finally, they should be sent for disposal—by land filling or burial. Alternatively, they can be sent to plastic industry for recycling, if facilities are available. They should never be incinerated or burnt as they contain halogenated polyvinyl chlorides that are known to emit 'dioxins' into the atmosphere which are highly carcinogenic
- The transparent puncture proof container containing sharps also need special treatment before disposal. The sharps should be
 - Destroyed before disposal
 - Disinfected by 1% sodium hypochlorite solution
- Sharps should be disposed in a sharp pit which is well covered and protected and not accessible to rag pickers for reuse
- Noninfectious waste (80–85%) generated in hospital can be collected in any conveniently available colour bag (green, black, white) and without any treatment can be sent to municipal garbage bins safely or can be used for vermicomposting or land filling (if land is available)

■ Describe the techniques available for treatment of infectious waste.

The following techniques are available for treatment of infectious material:

1. Double Chambered Incineration

- Incinerator burns waste at high temperature in order to reduce its volume. The double-chambered incinerator contains two chambers:
 - Primary chamber—in which the waste is burnt at 800°C
 - Secondary chamber—where combustion of volatiles and gases emitted from the primary chamber occurs and the gases are subjected to conditions of high temperature (1000°C) and excess air
- The negative pressure maintained inside the incinerator forces the end-gases out of the chimney

Uses.

This method is used for treatment of

1. Microbiological and pathological waste
2. Soiled dressings
3. Anatomical waste
4. Cytotoxic waste

Advantages

1. Ensures complete disposal of waste
2. Reduces volume of waste

Disadvantages

1. Generates highly toxic gases if PVC plastic material is present
2. Not recommended for sharps and plastic waste
3. High cost
4. Expensive to maintain and operate

2. Autoclaving/Steam Sterilization

- Autoclave relies on the circulation of steam through infectious waste to decontaminate it
- The following two kinds of autoclaves are available for this purpose:
 - The gravity displacement type
 - The prevacuum type
- In prevacuum type, steam is created outside the chamber loaded with waste
- Air in the chamber is then gradually removed as steam is injected in
- Temperature is reached about 134°C for 30 minutes and the waste in the bags is sterilized
- The prevacuum type tries to eliminate 'cold spots' and 'air pockets' (region where the steam is unable to penetrate) by creating vacuum and ensures complete elimination of air (only 5% air remains in the main chamber) because of this, there is quicker heating of the chamber and greater sterilization
- In the gravity displacement type about 25% of air remains in the main chamber, because of which heating is not quick—requires longer duration, and also sterilization is less efficient as compared to prevacuum type

Uses

Autoclaving is used for the treatment of

1. Microbiological and pathological waste
2. Blood and blood products

3. Body fluids
4. Sharps and plastic wastes

Advantages

1. Economical
2. Easy to operate
3. Makes waste completely noninfectious
4. Disinfects waste without hazardous emissions

Disadvantages

No significant reduction in waste volume.

3. Chemical Disinfection

- Several factors influence the effectiveness of chemical treatment. These are:
 - Type of disinfectant
 - Quantity and concentration of disinfectant
 - Contact time and temperature
- Chemicals such as formaldehyde (6–8%), glutaraldehyde, hydrogen peroxide (6–30%) and chlorine in various forms (most commonly as hypochlorite solution, 1%)—are commonly used for the treatment of waste

4. Microwaving

- This technology involves the use of radiation produced by the microwave to break apart molecular chemical bonds and thus disinfect infectious waste
- This process involves preshredding the waste, injecting it with steam in a special treatment chamber and evenly heating it for 25 minutes at 97°C–100°C under a series of microwave units (radiation spectrum between the frequencies of 300–300,000 MHz)

Uses

For the treatment of infectious waste.

5. Hydroclaving

- The hydroclave is an expansion of the autoclave technology
- There is indirect heating without any contact with the waste by introducing steam into the outer jacket while the waste is kept inside another chamber and turned mechanically with the help of a series of large rotating rods which spin continuously, rupturing the waste bags and ensuring complete exposure to heat
- The moisture content in the waste turns into steam and builds pressure inside the vessel
- Sterilization is carried out at 132°C for 15 minutes or at 121°C for 30 minutes
- This treatment results in fragmentation and dehydration with 80–85% reduction in volume and 65–70% reduction in weight
- After sterilization is completed, the waste is passed through a shredder, which renders the waste unrecognizable
- The waste after shredding can be recycled or land filled

Uses

Can be used for treatment of pathological and microbiological waste, sharps, animal wastes, etc.

Advantages

- No pretreatment of waste is required
- Complete fragmentation and dehydration is achieved
- Reliable for the treatment of pathological and highly infectious waste
- Reduction in volume and weight reduces the transportation and land filling costs
- Economical and simple for operation and maintenance

6. Plasma Technology

In this plasma torch is used to burn materials at very high temperature 2000°–3000°C, sometimes up to 10,000°C that allows complete destruction of waste.

Advantages

1. It causes transformation of all hydrocarbonated products into combustible gas without solid residues
2. No segregation of waste is needed

12

Chapter

Bacterial Genetics

■ Write a short note on the mode of genetic transmission of characters.

Gene is a segment of DNA (deoxyribonucleic acid) that carries information for a specific biochemical or physiological property in its nucleotide sequence. All hereditary characteristics are encoded in genes (DNA). Individual organisms transmit these characteristics to their offspring through genes. The chromosomal DNA plays an important role in the maintenance of characters constant from generation to generation, by its accurate replication. However, small proportion of heritable variations may occur in daughter cells.

■ Briefly describe the structure of DNA.

- DNA is the storehouse of genetic information
- DNA is double-stranded molecule consisting of complementary nucleotides
- The two strands are wrapped around each other to form a double helix (Watson and Crick model) (Fig. 12.1)
- Each strand has a backbone of deoxyribose sugar and phosphate group arranged alternately
- Each deoxyribose is attached to one of the four nitrogenous bases. These bases are
 - Adenine (A) and Guanine (G)—Purine bases
 - Thymine (T) and Cytosine (C)—Pyrimidine bases
- The purine 'A' is paired with pyrimidine 'T' and the purine 'G' is paired with pyrimidine 'C'
- The bases are paired by hydrogen bonding in the centre of the molecule. The hydrogen bonds help to hold two strands together. A-T is held by two hydrogen bonds and C-G by three hydrogen bonds
- The order of nitrogen base pairs is highly specific and contains the genetic information for the organism
- Each of the four bases is bonded to phospho-2'-deoxyribose to form a nucleotide (nucleoside—combination of a purine or pyrimidine plus a pentose sugar, it is without a phosphate group)
- In a molecule of DNA, the number of A is equal to T and number of C is equal to G, hence the base ratio of given DNA (A + T to G + C) is constant for each species but varies greatly from one species of bacterium to the other
- As the strands are complementary, if the sequence of bases in one strand is ATGC, the sequence of other strand is TACG
- During replication, each strand serves as a template for the synthesis of complementary strand. The two strands separate at one end and synthesize complementary strand with which it combines forming a new duplex (double helix), which is transmitted to the progeny with genetic information present in parent

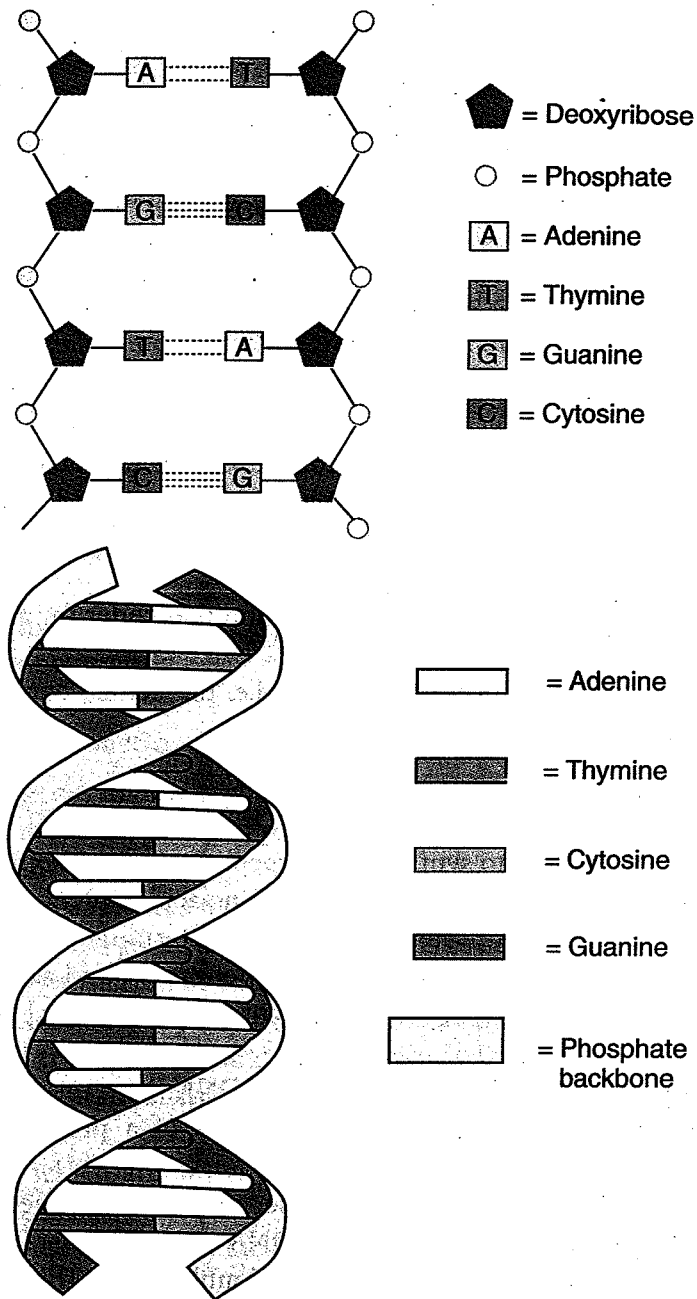


Fig. 12.1 Structure of DNA (double helix).

■ **Briefly describe the structure and functions of RNA.**

- RNA most frequently occurs in single-stranded chains
- It differs from DNA in the following ways:
 - It is single-stranded
 - It contains ribose sugar instead of deoxyribose
 - Instead of thymine (T), uracil is the base present in RNA
 - There are three different types of RNA in a cell—messenger RNA (mRNA), ribosomal RNA (rRNA) and transfer RNA (tRNA)
- Their functions are as follows:
 1. To communicate DNA genes sequence in the form of messenger RNA to ribosomes
 2. Ribosomal RNA (rRNA) present in the ribosomes translates messages into the amino acid structure of protein with the help of tRNA

■ **Define the following terms: (a) Gene, (b) Codon, (c) Nonsense codon, (d) Genotype and (e) Phenotype.**

(a) **Gene:** It is a segment of DNA (sequence of nucleotides in DNA) that codes or specifies for a particular polypeptide (functional product). A molecule of DNA consists of large number of genes; each gene contains hundreds of thousands of nucleotides. The length of a DNA is usually expressed in thousands of base pairs or kilo base (kb) pairs – 1 kb = 1000 base pairs. The bacterial chromosomal DNA is about 4000 kb. Each base pair is separated from the next by 0.34 nm so that the total length of chromosome is around 1mm (1000 μ).

(b) **Codon:** Genetic information is stored in the DNA as a code, the unit of which is a sequence of 3 bases—triplet. Each such triplet is known as a codon. Each codon specifies for a single amino acid, but more than one codon may exist for the same amino acid.

(c) **Nonsense codons:** Three codons—UAA, UGA and UAG do not code for any amino acid (do not form any amino acid) and act as 'stop codons' for terminating message for the synthesis of a polypeptide. These are known as nonsense codons.

(d) **Genotype:** It is the genetic make up, the information that codes for all peculiar characters of the organism.

(e) **Phenotype:** It is the manifestation of genotype, i.e. the actual, expressed structural and physiological properties of the organism.

■ **Elucidate the function of chromosomes in the synthesis of amino acids.**

- The function of chromosome is to synthesize polypeptide. Chromosome is functionally subdivided into segments, each segment determines amino acid sequence and hence a structure of discrete protein. The different proteins synthesized determine the structural and physiological properties of the organism by functioning as enzymes, and as components of membrane and other cell structures. The gene, a segment of chromosomal DNA, determines the structure of a distinct protein

- The way in which the genetic information in DNA is transcribed onto RNA and translated as the sequence of particular amino acids of a protein molecule occurs as follows:

- **Transcription:** An enzyme, RNA polymerase, attaches to the gene on DNA and synthesizes mRNA, using one of the strands in DNA as a template. This mRNA is complementary to one of the strands in the DNA double helix. This process is known as transcription

- **Translation:** The mRNA is then passed into cytoplasm where it attaches on the surface of rRNA. The mRNA possesses triplet base sequences known as codons. These codons are recognized by the anti-codon sequences of tRNA. The tRNA molecule contains a triplet of bases complementary to a triplet of bases on mRNA at one end and specific site for amino acid at the other end. The tRNA brings amino acid, attaches to complementary nucleotide triplet on mRNA and puts the amino acid into peptide linkage with the amino acid of neighbouring tRNA molecule. The ribosome moves along the mRNA until the entire mRNA molecule has been translated into corresponding sequence of amino acids. This process is known as translation

In this way the nucleotide sequence of the gene determines the structure of specific protein using mRNA as mediator.

- Give a brief account of extrachromosomal genetic elements—plasmids and episomes.

Plasmids

These are autonomously replicating, cyclic, double-stranded circular DNA molecules that are distinct from chromosomes. They are present in the cytoplasm of a bacterium in free state and carry genes for functions other than cell growth. These are of two types:

- Conjugative—large and self-transferable, e.g. R, F and certain bacteriocinogen plasmids
- Nonconjugative—small and nontransferable, but can be transduced—transferable with the help of conjugative plasmid, e.g. *r*-determinants and some bacteriocinogen plasmids

Episomes

- These are also extrachromosomal genetic material
- They are either integrated with chromosomal DNA of bacteria or exist autonomously in the cytoplasm
- They are similar to plasmids—not possible to differentiate from a plasmid; hence the two terms are used synonymously

- Enumerate the properties of plasmids.

- Plasmids contain genes for self-replication—**replicate autonomously**
- Two members of the **same group of plasmids cannot co-exist** in the same cells
- May **express phenotypically**
- Some plasmids have apparatus for their transfer—**self-transferable**, also they can mediate transfer of chromosomal genes and other plasmids by integration
- Have ability to get transferred by **transfection**, i.e. transfer without cell contact
- Some plasmids have ability to jump from chromosomal DNA to plasmid DNA or vice versa by a process called **transposition** and are known as transposons or jumping genes
- Plasmids are **lost spontaneously or isolated artificially** (cured) by curing agents (chemical agents)
- They carry genes, which are **not essential for the life and function of the host** but they confer various properties, such as
 - Fertility—attributed to fertility factor (F). The cell containing F-factor (plasmid) is F^+ and synthesizes sex pilus
 - Resistance—attributed to resistance plasmids (R-factors), which are a group of conjugative plasmids—large plasmids with R-factors. They possess two functionally different parts, which are:
 1. Resistance transfer factor (RTF)—contains genes for autonomous replication and conjugation
 2. Resistance determinant (*r*-det)—smaller component, determines drug resistance. A single factor may carry gene-controlling resistance to multiple antibiotics
 - Bacteriocin production—attributed to plasmids known as bacteriocinogen
 - Other properties are:
 1. Production of enterotoxin in *E. coli*
 2. Production of exotoxin
 3. Production of haemolysins
 4. Production of surface antigens
 5. Production of enzymes of special catabolic pathways

■ **What is meant by phenotype? Mention the phenotypic variations generally observed in bacteria.**

Phenotype

- Phenotype (*phaeno* meaning display) is expression of various characters of inherited traits as per genetic make-up by bacterial cells in a given environment

Phenotypic Variations

- A bacterial cell may exhibit certain phenotypic appearances in different environmental conditions
- Phenotypic changes are temporary to changed environmental conditions and are reversible when the original environmental condition is restored
- Some of the important phenotypic variations occurring in bacteria are:
 - **Synthesis of flagella**—*Salmonella typhi* is flagellated but loses its ability to synthesize flagella when cultured in phenol agar. This effect is again reversed when subcultured from phenol agar into broth containing no phenol
 - **Synthesis of enzymes**—synthesis of enzyme beta-galactosidase, necessary for lactose fermentation by *E. coli*, occurs only when it is grown in lactose containing medium. Though *E. coli* possesses the genetic information for the synthesis of beta-galactosidase, actual synthesis is induced by presence of lactose (inducible enzyme). This has led to development of operon concept by Jacob and Monod. The operon concept explains the regulatory mechanism which involves structural and regulatory genes
- Lactose fermentation in *E. coli* requires three enzymes, namely
 1. Beta-galactosidase, coded by structural gene—*lac Z*
 2. Galactosidase permease, coded by structural gene—*lac Y*
 3. Transacetylase, coded by structural gene—*lac A*
- These genes are arranged linearly in a sequence forming a functional unit, the *lac* operon
 - The regulatory gene *lac I*—codes for a repressor
 - In addition, there are promoter and operator regions, which lie between *lac I* and structural genes (Fig. 12.2)

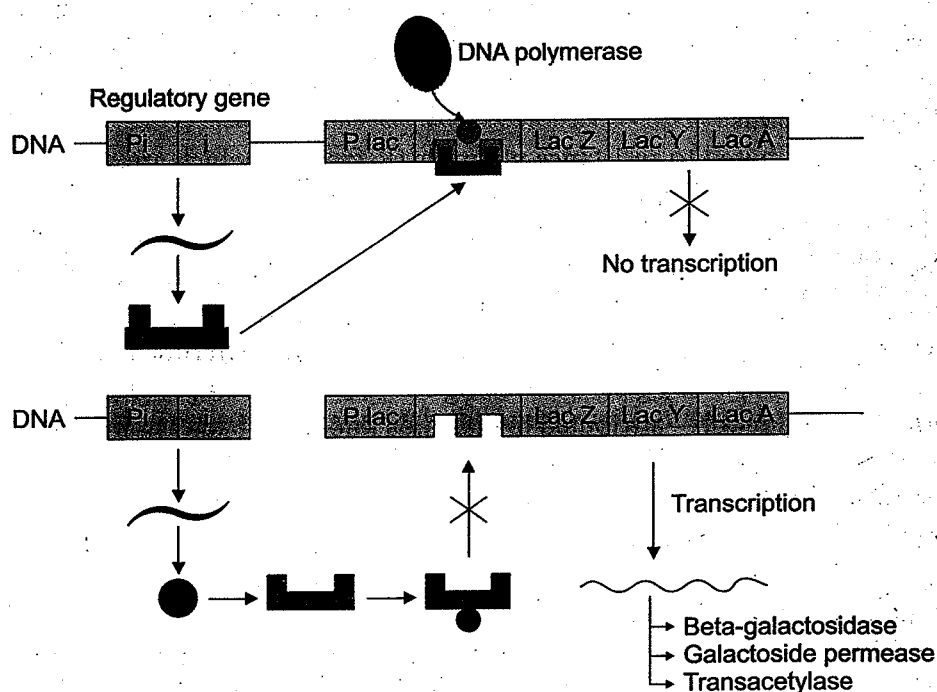


Fig. 12.2 Lac operon.

- The repressor can combine with the inducer molecule (lactose) when it is present in the medium, otherwise it combines with operator region and prevents the travel of RNA polymerase from the promoter region to the operon.
- The repressor molecule has affinity for lactose
- In presence of lactose, it leaves the operator free and allows RNA polymerase to travel along the structural genes in a sequence for transcription of RNA to synthesize enzymes necessary for lactose fermentation
- When the lactose is completely metabolized, the repressor again attaches to the operator and prevents travelling of RNA polymerase from the promoter region thereby inhibiting transcription and enzyme synthesis

■ **What is meant by 'genotype' of an organism? What are genotypic variations and how do they occur?**

Genotype

- Genotype refers to the genetic constitution of an organism. It is the sum total of the genes

Genotypic Variations

- These variations are due to changes in the genetic material and hence, genotypic variations are stable, heritable and not influenced by environment
- Genotypic variations occur by mutation or transmission of genetic material from one bacterium to another (gene transfer)

■ **Define the word mutation.**

Mutation is defined as a permanent alteration/change in the sequence of bases of DNA. This change may or may not lead to detectable change in the cell phenotype. When mutations are not expressed phenotypically, they are known as **silent mutations**. The different forms of gene produced by mutation are called **alleles**. Mutation may be spontaneous or induced by mutagenic agents.

■ **Write a short note on spontaneous mutations.**

Spontaneous mutations occur in the absence of any known mutagen and are caused by errors in base pairing during DNA replication. Spontaneous mutations for a given gene generally occur with a frequency of 10^{-8} – 10^{-6} in a population derived from a single bacterium.

Mutational changes include:

1. Nucleotide replacements
2. Deletions and insertions

Nucleotide Replacements

These are mutations occurring because of replacement of bases—**base pair substitution**. This occurs in the following two ways:

1. **Transition:** Replacement of a purine by another purine, or replacement of a pyrimidine by another pyrimidine, e.g. replacement of AT by GC
2. **Transversion:** Replacement of a pyrimidine by purine or vice versa, e.g. replacement of GC to CG

Deletions and Insertions

During DNA replication, there may be insertion of one or a few base pairs or deletion of one or a few base pairs. This insertion or deletion shifts the normal reading frame of the coded message, forming new set of triplet codon that specifies the incorrect amino acids. These mutations are called **frame shift mutations**.

- The appearance of bacteriophage-resistant mutants without prior contact with selective agent indicates that mutation has occurred

■ Write short notes on transformation and transduction.

Transformation

- It is the process in which the hereditary alteration in the properties of one bacterium is mediated by soluble DNA obtained from another bacterium
- The genetic information is transferred through free or naked DNA
- It was discovered by Griffith (1928) in pneumococci. He observed that when noncapsulated, rough strain is injected in mouse with heat killed, smooth strain (capsulated) of pneumococci the mouse dies and living smooth pneumococci are isolated from the blood of dead mouse. He guessed that heat-killed smooth strain might have liberated something which conferred on the rough strain the ability to make a new type of capsular polysaccharide and thus might have converted rough strain to smooth strains making them virulent. In 1943, Avery, MacLeod and McCarty identified the transforming principle as DNA

Mechanism

During transformation, the bacterium becomes competent and during this period of competence, the double-stranded DNA binds to sites (present during the stage of competence only). This DNA is enzymatically cleaved to form fragments and fragmented DNA is efficiently taken up and incorporated into the recipient DNA.

Significance

- It can be used for genetic mapping. To introduce DNA into bacterial cell that has been synthesized *in vitro*.

Transduction

- It is a process of gene transfer in which a portion of DNA from one bacterium is transferred to another by a bacteriophage (viruses that parasitize in bacteria). It not only transfers chromosomal DNA but may also transfer plasmid or episomal DNA
- It was discovered by Zinder and Lederberg in 1952 in *Salmonella*
- There are two types of transduction. These are as follows:
 1. **Generalized transduction:** It is the transduction in which any segment of DNA has a roughly equal chance of being incorporated into a phage coat
 2. **Specialized (restricted) transduction:** It is the transduction in which only a particular segment of DNA (particular genetic trait) is transduced by a bacteriophage

Mechanism

- Bacteriophage attacks the bacterium
- It releases its nucleic acid in the bacterial cell and starts its replication process
- During the maturation process of the virus particle a few phage heads may envelope any fragments of bacterial DNA in generalized transduction or it may envelope only a particular segment of bacterial DNA in specialized transduction along with phage DNA (Fig. 12.5)
- When such a phage infects another bacterium, it introduces bacterial DNA which gets incorporated into bacterial chromosome
- Thus, the transfer of bacterial genes to other bacterium occurs

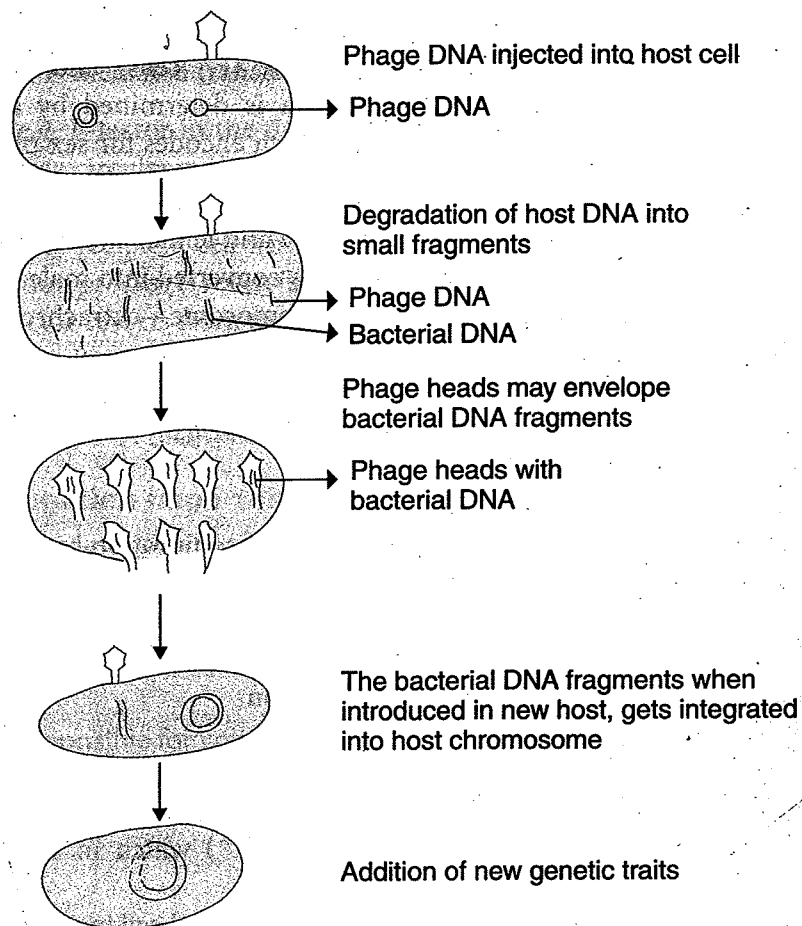


Fig. 12.5 Generalized transduction.

Significance

- It is an excellent tool for the genetic mapping of bacteria
- It can be used as a method of genetic engineering in the treatment of some inborn errors of metabolism

■ Explain lysogenic conversion.

In transduction, adsorption of bacteriophage is followed by injection of phage DNA into the host cell. If this phage is of virulent type—the result is lytic cycle in which new phage particles are synthesized, which are released by lysis of the host cell. But when the infecting phage is a temperate phage the result is **lysogenic conversion/lysogeny** in which phage DNA is incorporated into the host cell DNA as a prophage, which multiplies synchronously with the host cell DNA. It acts as an additional chromosomal element and the host acquires significant new characters, which are transferred to the progeny, e.g. the diphtheria bacilli are toxigenic because of the lysogenic bacteriophage— β -phage. Elimination of β -phage renders them nontoxigenic.

■ What is conjugation? Explain the mechanism and process of conjugation. Mention its significance.

Conjugation

It is the process of transfer of genetic material in which a 'male' or 'donor' bacterium mates or makes physical contact with the 'female' or 'recipient' bacterium and transfers genetic material to it.

It was discovered by Lederberg and Tatum (1946) in a strain of *E. coli* called K12.

Mechanism

During conjugation, one partner acts as 'donor'—male (F^+ cells) and the other acts as 'recipient'—female (F^- cell). The maleness in bacteria is determined by a plasmid called F factor (F for fertility)—it is a conjugative plasmid, which encodes for sex pilus necessary for conjugation.

- F-factor is transferred only by conjugation when male and female make cell-to-cell contact
- After the F-factor is transmitted, every female (F^-) is converted into male (F^+)
- 'F' is self-replicating, separate from the bacterial chromosome—extrachromosomal genetic material known as plasmid. It is one of the plasmids
- Plasmids not only determine maleness but also determine other characters. Many plasmids carry genes, which confer new properties on the host cell, e.g.
 - Resistance to drug—R-factor
 - Production of colicin—Col factor
- These plasmids carry genes, which govern the process of conjugation by acting as donors or male cells in conjugation—these are named as **transfer factor** or **conjugative plasmid**

Conjugation Process

- The cells come in direct contact through pilus retraction
- One strand of plasmid DNA is broken down
- The broken strand enters the recipient
- The complementary strands are synthesized in both donor and recipient
- This results in conversion of female (F^-) into male (F^+) which in turn conjugate with another female (F^-) (Fig. 12.6)
- Thus, the conjugation is a mechanism imposed on the bacterial cell by a plasmid, the normal result of which is the transfer of plasmid DNA. It may also mediate the transfer of chromosomal DNA in some bacteria

Significance

- Confers new properties in recipient cell—most important one is drug resistance property
- Useful for gene mapping
- Important in increasing genetic diversity

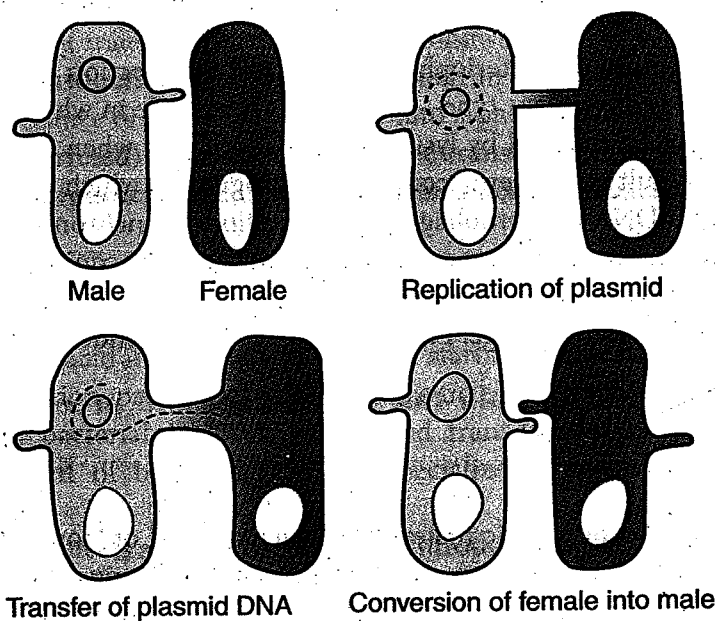


Fig. 12.6 Conjugation.

■ Explain the mechanism of F-mediated chromosome transfer in Hfr cell.

The mechanism of F-mediated transfer of chromosomes in Hfr cell is as follows:

- The plasmid, e.g. F-factor in the donor cell gets integrated with the bacterial chromosome forming one large circular molecule. The cell in which this occurs and the clones which arise from them are called **Hfr cells** (high frequency of recombination). These cells transfer chromosomal DNA to recipient cell with high frequency
- As this integration process is reversible, the conversion of F^+ cell into Hfr cell is also reversible
- When the F-factor reverts from the integrated state to free state by detaching from chromosome, sometimes, it may carry some chromosomal DNA with it. This F-factor carrying some chromosomal DNA is known as F' and when this F' cell conjugates with F cell, it transfers the chromosomal DNA incorporated with it. This process is known as **sexduction**

■ How is conjugation important to medical science?

The two medically important plasmids, **colicinogenic (Col) factor** and **resistance transfer factor (RTF)**, which confer properties of colicin production and resistance to drug are transferred by conjugation.

Col factor

- It is plasmid responsible for colicin production in *E. coli*
- Colicin is an antibiotic-like substance lethal to other enterobacteria
- This plasmid (Col factor) is transferred by conjugation

RTF

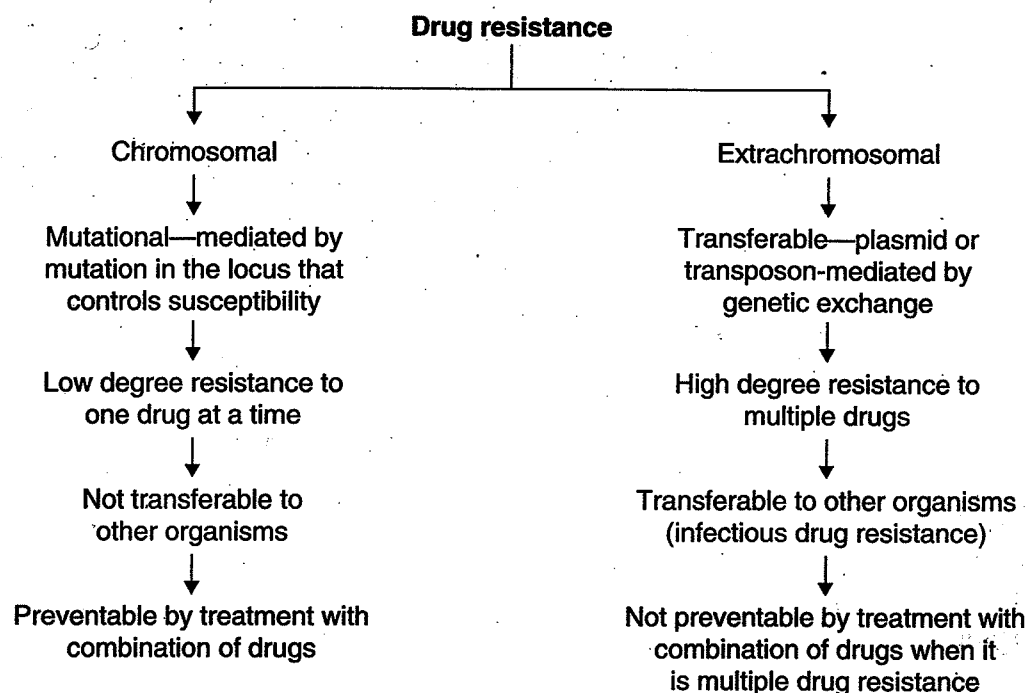
- It is a plasmid responsible for spread of multiple drug resistance among bacteria
- RTF and *r* (resistance determinant) are two components of the plasmid known as an R-factor
- The RTF mediates a conjugational transfer of *r* determinant, which carry genes for resistance to drug
- An R-factor may have many *r* determinants, and therefore resistance to many drugs can be transferred simultaneously
- This is a very common mode of drug resistance particularly in enteric bacteria
- In addition to transfer of drug resistance, RTF is also shown to mediate conjugal transfer of genes responsible for enterotoxin and haemolysin production in some enteropathogenic *E. coli*

■ Mention the genetic mechanism of acquiring drug resistance. Diagrammatically represent the genetic basis of drug resistance.

- The drug resistance is acquired after a change in DNA
- This change may occur by alteration in the structure of chromosome (called mutation) or by acquisition of extrachromosomal DNA which is the result of genetic exchange, in which the sensitive organism receives extrachromosomal genetic material (part of DNA) offering resistance to one or multiple drugs from resistant organism (see Flowchart 12.1)

■ What is mutational drug resistance? Giving an example, explain how it is important in the treatment of bacterial infections.

- **Mutational drug resistance** arises as a result of spontaneous mutation in a locus of chromosomal gene that controls susceptibility to a drug. The presence of a drug serves as a selective mechanism to suppress susceptibility and promote the growth of drug resistant mutants
- In clinical practice, mutational resistance is very important in tuberculosis. If only one drug is given to the patient, tubercle bacilli die initially in large numbers but soon resistant mutants emerge and multiply unchecked. A serious consequence of unchecked drug resistance



Flowchart 12.1 Genetic basis of drug resistance.

is emergence of multiple drug resistant strains. Hence, to avoid emergence of resistance by mutation, a combination of four drugs is used. By using four drugs, a mutant resistant to one drug will be killed by the other drugs as mutational drug resistance involves one drug only at a time

■ **Write a short note on transferable (infectious) drug resistance.**

- Transferable (infectious) drug resistance arises as a result of transfer of extrachromosomal genetic material (plasmid or episome) through the process of conjugation or transduction
- Transfer of resistance against single or multiple drugs (most common) through plasmid known as R-factor is a very important method of drug resistance
- The R-factor contains following two components:
 - r-determinant—determines resistance to drug
 - RTF—mediates transfer of r-determinant
- The transferable drug resistance is very common in Enterobacteriaceae
- Resistance in *Staphylococcus aureus* to penicillin is due to a plasmid that produces penicillinase. This plasmid is devoid of RTF and its transfer from resistant to susceptible strain occurs by transduction rather than conjugation

■ **Write a short note on transposable genetic material.**

- **Transposons (Tn)** are DNA sequences, which have ability to move from one plasmid to another or from plasmid to chromosome and vice versa. Due to this property, they are also known as **jumping genes**
- Because they are incapable of autonomous existence, they are transferred from one DNA molecule to another. This process is known as **transposition**, which is different from recombination, as it does not require any DNA homology between transposable element and the site of insertion in DNA molecule
- Transposons are larger segments of DNA about 4–25 kb with one or more genes in the centre and the two ends carrying inverted repeat sequences complementary to each other but in

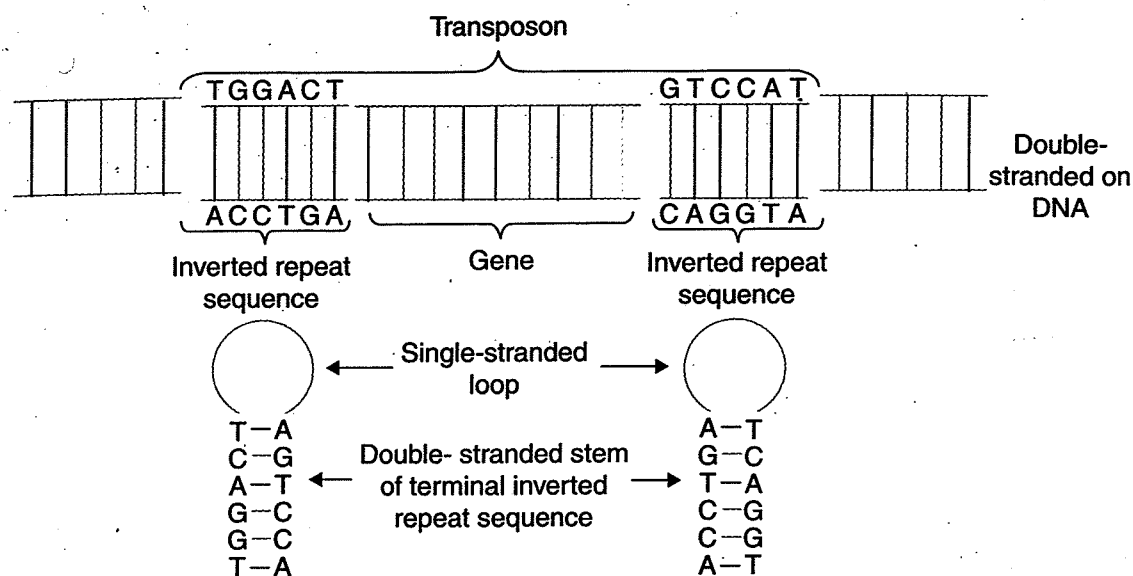


Fig. 12.7 Structure of transposons.

the reverse order. Because of this, each strand of transposon can form a single-stranded loop carrying the gene or genes and a double-stranded stem formed by hydrogen bonding between the terminal inverted repeat sequences (Fig. 12.7)

- The gene/s contained in a transposon encode/s for at least one function—most often for antibiotic resistance and may also encode for toxin production
- Another group of DNA sequences, which are smaller in size (1–2 kb) and have ability to move from one DNA molecule to another genetic material are known as **insertion sequences (Is)**
- These elements (Tn and Is) can transpose DNA blocks to chromosome, plasmid or phage DNA and can subsequently transfer the DNA blocks to other cells and new characters thereby
- They are now recognized to play an important role in bringing about various types of mutations in the chromosomes

■ **What is genetic engineering/DNA recombinant technology? With the help of a diagram explain the various steps involved in it.**

Genetic Engineering/DNA Recombinant Technology

- It is artificial manipulation of DNA to identify and derive useful genes and genetic product
- Through this technique it is possible to isolate the desired genes from one kind of organism and introduce into another organism changing its genetic make-up and to make it to acquire new property in addition to its original one
- The central process in DNA recombinant technology is gene cloning which involves
 - Production of defined fragment of DNA
 - Its propagation and duplication in a suitable host

Steps

1. Generation of DNA Fragments

The first step is construction/generation of recombinant DNA molecule with the help of enzymes called restriction endonucleases, which recognize specific sequence of nucleotides in DNA and cleave them into fragments containing desired genes, which are isolated for further processing.

2. Cloning Vectors

Cloning vectors are needed to insert foreign DNA. A vector is a DNA molecule in which a DNA fragment is cloned. For propagation of DNA fragments, it must be joined to a vector to insert a foreign DNA into a cell. Vectors most commonly used are:

- Plasmids—such as R-factor
- Temperate bacteriophages
- Cosmid—a hybrid vector containing both plasmid and phage

3. Joining of DNA Molecules

The final step in the construction of a recombinant DNA molecule is the joining together of a particular DNA fragment of interest to the vector molecule. This process is known as ligation and is catalyzed by an enzyme DNA ligase.

4. Introduction of Recombinant DNA into Host Cell

The recombinant DNA molecule prepared *in vitro* is then introduced into suitable host, i.e. cloning organism by transformation. *E. coli* is most commonly used for this purpose. In addition to it, other bacteria and vaccinia virus can also be used.

5. Detection and Characterization of Recombinants

Recombinant DNA (cloned genes) in host cell is detected and characterized by hybridization and immunological methods. It can also be detected by the detection of products of recombinant DNA.

6. Obtaining the Desired Product

The host cell containing recombinant molecule is grown in a suitable medium and the host cell growth carrying specified genes are produced and the desired protein is obtained in large quantities by growing the host cell (Fig. 12.8).

■ List the various applications of genetic engineering.

The technique can be used for the following purposes:

1. **Production of hormones**
 - a. Growth hormones
 - b. Insulin
 - c. Thymosin-alpha-1—used in brain and lung cancer
2. **Production of vaccines**
 - a. Hepatitis B vaccine
 - b. Rabies vaccine
 - c. Malaria vaccine (presently under experimental stage)
 - d. HIV capsid protein (under trial)
3. **Production of enzymes** such as urokinase—used to dissolve blood clots
4. **Production of interferon**—for the treatment of viral diseases and certain types of cancer
5. **Production of antimicrobial agents**
6. **Production of proteins** of therapeutic interest, e.g.
 - a. Interleukin-2
 - b. Tumour necrosis factor
 - c. Factor VIII
7. It may be possible in future to alter the genetic material in human egg and treat inherited diseases like haemophilia, thalassaemia, sickle cell anaemia, etc. (gene therapy)

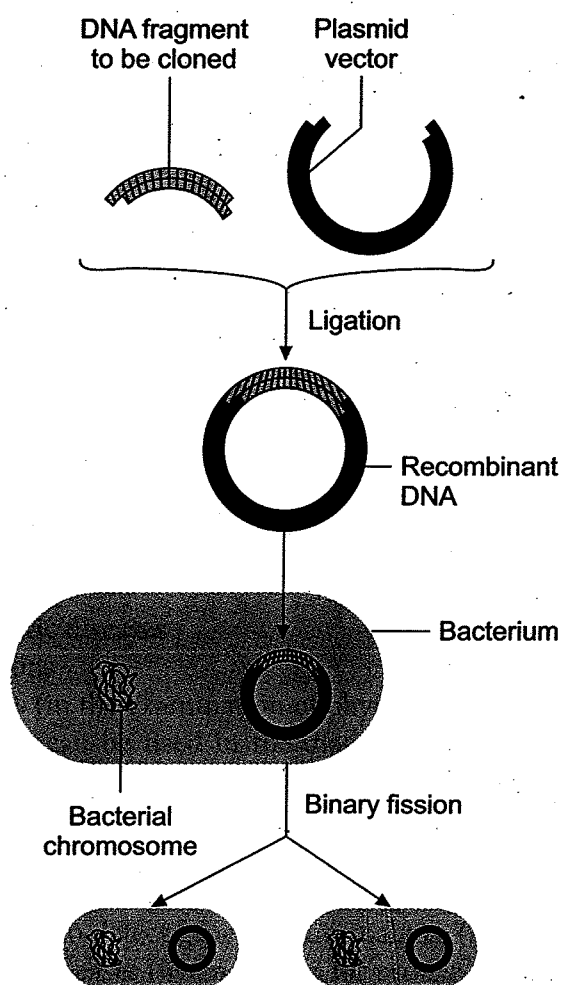


Fig. 12.8 Gene cloning and recombinant DNA technique.

■ **What are DNA probes? Describe in short the procedure, advantages and applications of DNA probes. Also list the commercially available DNA probes.**

- DNA probes are radiolabelled or chromatographically labelled pieces of single-stranded DNA, which can be used for the detection of homologous DNA
- The technique is based upon hybridization of test DNA (DNA to be detected in a test sample/culture) with DNA probe
- Hybridization is the technique in which two single strands of nucleic acid come together to form a stable double-stranded molecule
- If the test DNA is present in the sample, it will conjugate with the DNA probe forming a stable double-stranded DNA
- All microorganisms contain some unique sequences of nucleic acid within their genome that make them different from all other organisms
- This unique sequence of nucleic acid can be recognized by hybridization with a probe, which is unique for that particular organism
- Various diagnostic DNA probes have been developed for identification of different microorganisms

Procedure

- Nucleic acid of the test pathogen is extracted and denatured before hybridization with probes
- DNA probes containing unique sequences are added
- The double-stranded DNA formed by hybridization is then separated from the rest of the labelled probe DNA and sample

- The amount of probe bound to sample is recorded and the degree of binding is compared with positive and negative controls and results are interpreted

Applications

- To detect microbes that are difficult or impossible to culture
- To detect microbes which do not have diagnostic antigens
- To differentiate virulent and avirulent strains
- To detect latent viral infection
- For rapid confirmation of cultured organisms
- To identify antibiotic resistance genes
- To develop epidemiological markers

Commercially available DNA probes

The following probes have been commercially developed:

1. *Legionella pneumophila*
2. *Campylobacter jejuni*
3. *M. tuberculosis*
4. *H. pylori*
5. *M. avium*
6. *M. intracellulare*
7. *E. coli* (LT and ST toxins)
8. Herpes simplex virus
9. Hepatitis B virus
10. Rotavirus
11. HIV
12. *Plasmodium falciparum*

■ Write a short note on polymerase chain reaction (PCR).

History

Developed in 1983 by Kary Mullis (awarded Nobel Prize in 1993).

Principle

It is an *in vitro* method of producing large amounts of specific DNA fragments of defined length and sequence from small amounts of complex template. It amplifies a specific DNA sequence (or gene) of interest.

Procedure

It involves the following three steps:

1. Melting of DNA at 94°C to convert double-stranded DNA to single-stranded DNA
2. Annealing of primers to target DNA at 50°–70°C
3. Synthesis of DNA by addition of nucleotides from primers by action of DNA polymerase
4. Melting of the newly formed DNA and repeating the process again and again; an exponential increase in the amount of DNA occurs (Fig. 12.9)

RNA PCR

It is a modification of PCR technique in which RNA template is used for amplification. From this RNA template, a complementary copy of DNA is obtained by using reverse transcriptase and then the routine method of PCR is used which amplifies cDNA (complementary DNA).

Applications of PCR

- It is a rapid method (one day), has number of applications

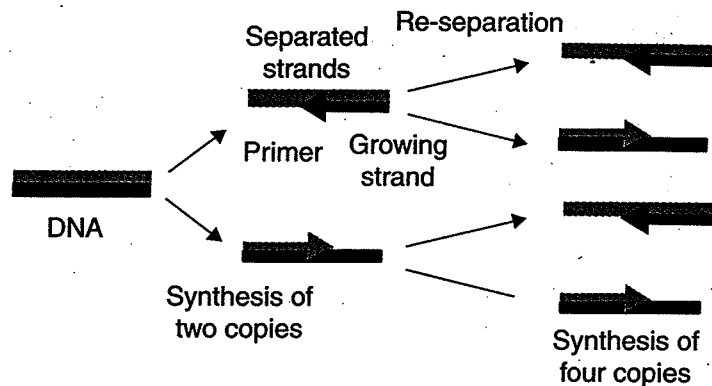


Fig. 12.9 PCR.

- It is a versatile tool useful in
 - Infectious diseases
 - Genetic and neoplastic diseases
 - Forensic investigations
 - Evolutionary studies (in the examination of phylogenetic relationships)
- In clinical laboratory, it can be used in the diagnosis of large number of bacterial, viral, parasitic and fungal diseases

■ Discuss blotting techniques.

Blotting techniques are of three kinds: southern blotting, northern blotting and western blotting. They are generally used to detect genetic abnormalities (when blotted for DNA or RNA), as well as certain infectious diseases (when blotted for protein).

Southern Blotting

The technique was developed by E.M. Southern and is named after him.

Procedure

- DNA fragments are obtained by digestion with restriction enzyme
- Digested DNA fragments are separated by gel electrophoresis
- Fragments are transferred by blotting on nitrocellulose or nylon membrane, which can bind the DNA
- The membrane bound DNA is converted to single-stranded form and treated with radioactive single-stranded DNA probes
- This results in hybridization, forming radioactive double-stranded segments, which can be detected on X-ray films
- This is DNA:DNA hybridization, used for **identification of DNA fragments**

Northern Blotting

- The technique of Northern blotting is similar to Southern blotting technique, but is used for **analysis of RNA**
- In this method, the RNA is separated by gel electrophoresis, blotted and identified using labelled probes

Western Blotting

- This is a technique used for **identification of proteins**
- The steps are same as in Southern blotting except for the probe used
- The probe used is specific radiolabelled or enzyme labelled antibodies
- The test is used for confirmation of HIV antibodies

13

Chapter

Infection

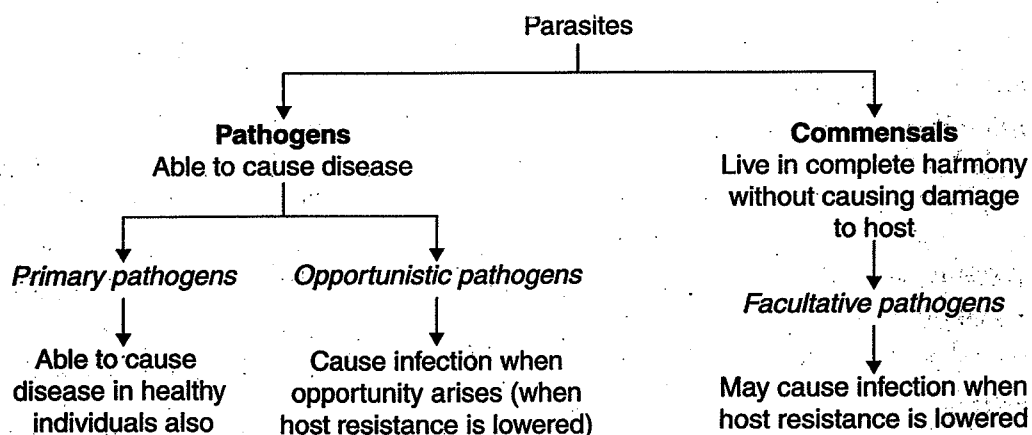
■ What is 'infection' and how is it different from 'disease'?

Infection is the biological process, which takes place in the body of macroorganism following the penetration of pathogenic microorganism. This penetration may result in obvious or latent disease or in a carrier state. Thus, infection is successful invasion (lodgement and multiplication) of the host tissue by an organism that may or may not lead to disease. Disease is a damage or injury produced by microorganism that impairs host function. It is not synonymous with infection.

■ Classify microorganisms based on host-parasite relationships.

Infection and immunity involve interaction between the host and the infecting microorganism. Based on their relationship with host, microorganisms can be classified as

1. **Saprophytes:** These are free-living microorganisms, which live on dead or decaying organic matter. They are found in soil and water, and play important role in degradation of organic matter. They are unable to multiply on living tissue, but sometimes, may produce infection when host resistance is lowered, e.g. *B. subtilis* may produce infection in devitalized host tissue
2. **Parasites:** Microorganisms that can enter and multiply in hosts are called parasites. The parasite may be pathogens or commensals (Flowchart 13.1)



Flowchart 13.1 Types of parasites.

■ Name and define various types of infections.

- **Primary Infection:** It is initial infection or fresh infection caused by microorganism
- **Reinfections:** Subsequent infections by the same pathogen in the same host are termed reinfections
- **Secondary Infection:** Infection by a new parasite, in a host whose resistance is lowered by pre-existing infectious disease, is termed secondary infection

- **Mixed Infection:** When more than one microorganism causes infection simultaneously it is called mixed infection
- **Focal Infection:** Localized infections or sepsis such as sinusitis, appendicitis and tonsillitis producing generalized effects such as vague physical or mental disturbances and allergic reactions are termed focal infections
- **Cross Infection:** In a person already suffering from a disease, when a new parasite sets up a new infection from another external source is termed cross infection
- **Nosocomial Infections:** Cross infections occurring in hospitalized patients are termed nosocomial or hospital acquired infections
- **Iatrogenic Infections:** Infections acquired during diagnostic or therapeutic procedures are known as iatrogenic or physician induced infections

■ **Classify infections based on (a) Clinical effects they produce, and (b) Source.**

(a) Classification of Infection Based on Clinical Effects

- **Inapparent Infections:** Infections in which clinical effects are not apparent. They are also known as subclinical infections
- **Atypical Infections:** Infections in which typical or characteristic manifestations of a particular disease are not present are called atypical infections
- **Latent Infections:** These are infections in which parasite remains in the tissue in a latent or hidden form that proliferate and produce clinical disease when host resistance is lowered

(b) Classification of Infection Based on Source

- **Endogenous Infections:** These are infections in which the source is from the host's own body. They are also known as autoinfections
- **Exogenous Infections:** Infections in which source of infection is external and not from the host's own body are known as exogenous infections

■ **Mention the various sources of human infections.**

Human beings may acquire infection from the following sources:

1. Humans

- A human being is the commonest source
- Parasite may originate from patient or carrier
- Carrier is a person who harbours pathogen without suffering from disease caused by it

Types of Carriers

- **Healthy carrier:** One who harbours pathogen but has never suffered from the disease caused by it
- **Convalescent carrier:** One who has recovered from the disease but continues to harbour the pathogen in his body
- **Temporary carrier:** One who carries organisms for short duration, i.e. less than 6 months
- **Chronic carrier:** One who carries organisms for long duration. May carry organisms for many years
- **Contact carrier:** One who acquires organisms from a patient
- **Paradoxical carrier:** One who acquires organisms from another carrier
- **Precocious carrier:** One who carries organisms during incubation period of the disease

Persons suffering from latent or subclinical infections are important sources.

Patient	→	Carrier	→	Susceptible individual
---------	---	---------	---	------------------------

2. Animals

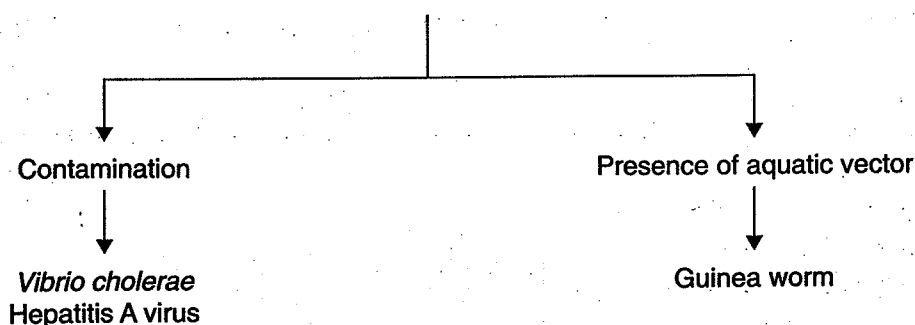
- Certain pathogens are able to cause infection in both humans and animals
- Hence, animals may act as a source of infection of such pathogens
- Infectious diseases transmitted from animals to man are called **zoonoses**.
- Infection from animal to man are transmitted by
 - Contact with animal
 - Animal bite
 - Ingestion of milk or meat
- In some animals—infection may be asymptomatic, such animals maintain pathogen in nature and may serve as reservoir for human infections—**reservoir hosts**
- *Examples of zoonoses are: plague, rabies, hydatid disease, brucellosis, etc.*

3. Insects

- Insects such as mosquitoes, ticks, mites, flies, fleas, lice, bugs, etc. may transmit pathogen to human beings
- Insects that transmit infections are known as vectors and diseases that are transmitted by insects are known as **arthropod borne diseases**
- There are two types of vectors, viz.
 - **Mechanical vectors:** They carry organisms on their legs, wings or body and transmit organisms to eatables, which then act as a source of infection, e.g. transmission of shigellosis and salmonellosis by domestic fly. Here no development of pathogen occurs during transmission
 - **Biological vectors:** They carry organisms in their body. Organisms undergo development in the body of the insect with or without multiplication, e.g. malarial parasite in mosquitoes
- The interval of time required for the biological vector to become infective is termed **extrinsic incubation period** (beginning from the time of entry of pathogen)
- Some vectors may act as reservoir host, e.g. ticks in relapsing fever and spotted fever

4. Soil and Water

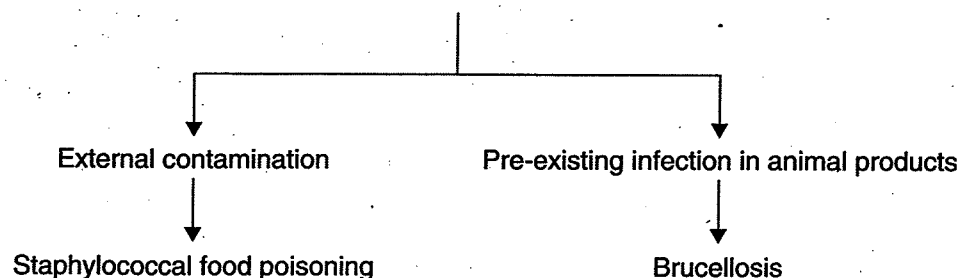
- Soil may serve as a source of infection of microorganisms which are able to survive in soil for long periods, e.g.
 - Fungal infections such as *Histoplasma capsulatum*
 - Parasitic infections such as Roundworm and Hookworm
 - Bacterial infections such as *Cl. tetani*—spores survive in soil for a long time, *Nocardia* species, etc.
- Water may act as source of infection either due to



5. Food

Contaminated food may be a source of infection.

Presence of pathogens in food may be due to



■ Describe the various routes by which transmission occurs.

Transmission occurs by the following routes:

1. Transmission Through Air (Inhalation)

- The organisms in mouth and respiratory tract are expelled out in the form of droplets during
 - Talking
 - Coughing
 - Sneezing is most dangerous (10^5 – 10^6 organisms/sneeze)
- The fate of droplet depends upon size. Droplets greater than $100\ \mu$ fall to the ground and dry. Pathogens resistant to drying remain viable in dust and cause infection
- Droplets smaller than $100\ \mu$ evaporate to form minute particles called droplet nuclei (1 – $10\ \mu$) and remain air borne for 1–2 hours acting as source of infection
- Infections transmitted by this route are:
 - Tuberculosis
 - Influenza
 - Measles
 - Mumps

2. Contact

It is of two types—Direct and Indirect

- **Direct contact**, i.e. sexual intercourse, kissing or close bodily contact. This route transmits contagious diseases such as syphilis and gonorrhoea
- **Indirect contact**, i.e. through the agency of fomites. Fomites are inanimate objects such as clothing, bedding, pencils or toys, which may be contaminated with pathogen from one person and act as a vehicle for its transmission to another person, e.g.
 - Transmission of diphtheria in school children by sharing of pencils
 - Transmission of trachoma by sharing of face towels

3. Ingestion

- Gastro-intestinal tract infections are generally transmitted by ingestion of contaminated food or water
- Infections transmitted by ingestion may be
 - Water borne, e.g. cholera
 - Food borne, e.g. food poisoning
 - Hand borne, e.g. dysentery
- In hand borne infection, hands contaminated with organisms in faecal matter is transmitted during feeding, if hands are not properly washed

4. Insects

- Insects may act as vectors and transmit infections from one person to another
- The transmission may be
 - **Mechanical**—direct transmission without development of microorganisms, with the help of legs, wings or body, e.g. dysentery
 - **Biological**—transmission during which there is development of pathogen in the body of insect, e.g. transmission of malaria

5. Inoculation

- Transmission of pathogen in some infections may occur by direct inoculation of causative agent in tissue, e.g.
 - Transmission of rabies virus by dog bite
 - Transmission of arboviruses by insect vectors
- Infection by inoculation may be iatrogenic when unsterile syringes and surgical equipments are used, e.g. HIV and hepatitis B virus may be transmitted by transfusion of infected blood or by using contaminated syringes and needles

6. Iatrogenic Transfer

- Transmission of infection may sometimes occur during diagnostic or therapeutic procedures such as:
 - Catheterization
 - Injection
 - Lumbar puncture
 - Dialysis, etc.
- This type of transfer is known as iatrogenic transfer

7. Congenital Transfer

- Transmission of infection from mother to fetus *in utero* by crossing the placental barrier is known as congenital or vertical transfer. Pathogens such as *Treponema pallidum*, HIV, Rubella virus, *Plasmodium* spp. are able to cross the placental barrier and infect fetus *in utero*.

■ What is microbial pathogenicity? Differentiate between pathogenicity and virulence.

- The capacity of microorganism to produce disease or tissue injury is known as pathogenicity/virulence
- Pathogenicity is the ability of the pathogen to inflict damage on the host, however, virulence is the degree of pathogenicity produced by pathogen. It is the quantitative measure of pathogenicity. As pathogenicity varies markedly from pathogen to pathogen, virulence is the term used for disease-producing ability of individual pathogens
 - The term pathogenicity is used for the ability of microbial species to produce disease, e.g. *Mycobacterium tuberculosis* is pathogenic
 - The term virulence is used for the ability of microbial strains to produce disease, e.g. pathogenic species of *M. tuberculosis* contains strains of varying degrees of virulence, including those, which are avirulent

■ Describe the factors predisposing to pathogenicity (virulence factors).

Factors predisposing to pathogenicity are as follows:

1. Adhesion

- Ability to adhere to host cell surface, usually epithelial cells, is an important virulence factor for extracellular pathogen
- Adhesion is a complex process involving specific components of microbial cells and receptor site on the host cell surface
- The structures on microbial cells that mediate adhesion are known as adhesins or ligands—these are specific surface molecules that interact with host cell surface. These include:
 - Pili or fimbriae in piliated or fimbriated organisms
 - Glycocalyx—a loose network of polymer fibres (macromolecules—polysaccharides synthesized and secreted by bacteria) extending outward from a cell
 - Lectin or other cell components
- Loss of adhesins makes the strain avirulent

2. Invasiveness

- The capacity of microorganism to invade and multiply in healthy tissue is invasiveness
- The capacity of nontoxic organisms to produce disease depends upon the quality of invasiveness
- There are two categories of bacteria which are invasive. These are as follows:
 - Some organisms like Gram-positive cocci initially attract phagocytic cells by chemotactic mechanism but resist phagocytosis and spread in tissue, e.g. pneumococci with the help of capsular polysaccharide resist phagocytosis—a major defense mechanism used by the host to prevent invasion and invade lung tissue causing extensive damage—leading to pneumonia
 - Some organisms like *M. tuberculosis*, *Salmonella* spp. and *Brucella* spp. are engulfed by phagocytic cell but resistant to phagocytic killing and spread with the help of phagocytic mobility to various parts of the body

3. Toxicogenicity

- It is the ability of an organism to cause disease by producing toxin that inhibits host cell function or kills host cell
- Bacteria produce two types of toxins
 - Endotoxins
 - Exotoxins
- The lipopolysaccharide (LPS) in the outer layer of cell wall of Gram-negative bacteria can cause
 - Hypotension
 - Shock
 - Fever
 - Disseminated intravascular coagulation (DIC)
 - Death
- Because of this toxicity and as they are incorporated within the cell wall, they are called endotoxins
- Exotoxins, by contrast, are not the structural components of the cell and are released from bacteria into surrounding media
- The important properties of endotoxins and exotoxins are as listed in Table 13.1

Table 13.1 Characteristics of endotoxins and exotoxins

Endotoxins	Exotoxins
Integral part of the outer layer of cell wall	They are not structural components and are released into surrounding medium
Chemical nature-complex containing phospholipid-polysaccharide-protein	Protein in nature
Heat stable	Heat labile. Readily inactivated by heat at 65°C in a few minutes
Toxic but less potent	Highly potent
Less specific in their effect	More specific in their effect, e.g. toxin of <i>V. cholerae</i> —enterotoxic, <i>Cl. welchii</i> —haemolytic, <i>Cl. tetani</i> —neurotoxic
Cannot be toxoid	Treatment with formalin or heat converts it into inactive form known as toxoid , which is nontoxic but antigenic and forms antitoxin which specifically reacts with the toxin and neutralizes it
Weakly antigenic	Highly antigenic
Produced by Gram-negative bacteria	Produced by Gram-positive as well as Gram-negative bacteria, e.g. <i>Corynebacteria</i> , <i>Bacillus anthracis</i> , <i>Clostridia</i> , <i>Staphylococci</i> , <i>E. coli</i> , <i>V. cholerae</i> , <i>Shigella</i> , <i>Bordetella</i> spp., etc.

4. Capsule

- The virulence of organism is influenced by nontoxic polysaccharide material in capsule
- Capsule serves as a physico-chemical barrier and prevents phagocytosis and helps to spread in the body
- It also acts as a barrier against the action of antibodies and increases the invasive property
- It may also help to resist the action of lysosomal enzymes

5. Other Virulence Factors

- A number of pathogens produce extracellular proteins that help in the establishment and maintenance of disease, most of them are enzymes. These include:
 - **Coagulase**—an enzyme produced by *Staph. aureus* forms fibrin barrier around the bacterium that helps to prevent the attack by host cells
 - **Fibrinolysin**—an enzyme (also known as fibrinolytic enzyme) that promotes the spread of infection by breaking down the fibrin barrier in tissues; fibrin clots are often formed at the site of infection to limit the infection to a small region of the body
 - **Hyaluronidase**—an enzyme produced by a streptococcus that breaks down hyaluronic acid (a polysaccharide present in connective tissue functioning as intracellular cementing substance) and promotes spreading of organisms in tissues
 - **Leucocidin**—produced by some bacteria that cause damage to polymorphonuclear cells
 - **IgA protease**—produced by gonococci and anaerobes, acts on IgA antibody, which plays an important role in local immunity
 - **Collagenase**—produced by *Cl. welchii*, breaks down collagen network supporting the connective tissue and contributes in spread of infection
 - **Other enzymes** such as proteases, nucleases and lipases that serve to depolymerize host proteins, nucleic acids and lipids and enzymes such as catalase, peroxidase, superoxide dismutase, heparinase, produced by various organisms contribute to pathogenicity

6. Infecting Dose

- To produce successful infection entry of an adequate number of bacteria (dose) is the important requirement
- The dosage may be estimated as
 - Minimum infecting dose (MID)—The minimum number of bacteria required to produce clinical infection in a susceptible animal under standard conditions
 - Minimum lethal dose (MLD)—The minimum number of bacteria required to produce death in animal under standard conditions
 - As animals exhibit considerable variations in susceptibility, these doses are more correctly estimated as
 - ID-50—Dose required to infect 50% of the animals tested under standard conditions
 - LD-50—Dose required to kill 50% of the animals tested under standard conditions

7. Route of Infection

Certain organisms are infective only when they enter through optimal route, e.g. organisms like *V. cholerae* are effective only by oral route but unable to cause infection by other routes. However, organisms like staphylococci and streptococci can initiate infection by any route.

■ Broadly classify infectious diseases based on the extent of infection.

Infectious diseases may be localized or generalized

- **Localized**—infection may be superficial or deep
- **Generalized**—localized infection may become generalized when immune system fails to check them
 - **Bacteraemia:** It is the circulation of bacteria in blood. Transient bacteraemia is frequently seen even in healthy individuals and may occur during chewing, brushing of teeth and straining at stools. The bacteria are immediately killed and are unable to initiate infection. Bacteraemia of longer duration occurs during generalized infections, e.g. typhoid fever
 - **Septicaemia:** When bacteria circulate, multiply and form toxic products in blood then it is called septicaemia
 - **Pyaeamia:** It is septicaemia caused by pyogenic bacteria with multiple abscesses in internal organs such as spleen, liver, kidneys, etc.

■ Classify infectious diseases based on their spread in the community.

Based on the spread of infectious diseases in community, they are classified as

- **Endemic**—diseases which are constantly present in particular area. The disease has special predilection for particular area
- **Epidemic**—when the disease spreads rapidly and attacks a large number of members of community. A similar condition amongst the animals is called epizootic
- **Sporadic**—a few scattered cases that occur in later part of an epidemic when the virulence of organism is low and the resistance of the population is high
- **Pandemic**—when a large number of persons are affected in many countries, within a short period then it is called pandemic
- **Prosodemic**—epidemic disease, which is smoldering and often transmitted from person to person chiefly by contact

14

Chapter

Normal Microbial Flora

■ Explain briefly what is normal microbial flora.

- A variety of microorganisms are normally present on and in our body. The population of microorganisms regularly present is known as the 'natural', 'indigenous', or 'normal flora' or 'normal micro-biota'. The normal flora is the part and parcel of the host and includes:
 - Saprophytes
 - Commensals
 - Facultative pathogens
 - True pathogens (occasionally)
- Most microorganisms normally present are commensals that exist in a symbiotic association with the host and obtain their nutrients from the secretions and waste products of the body
- Most of the microorganisms of normal flora are generally nonpathogenic but some may become pathogenic under certain conditions and may cause disease when they get opportunity, especially when the immunity is compromised in diseases like diabetes, leukaemia, immunodeficiency, AIDS, etc. Immunosuppressive therapy, instrumentation and surgery, burns, etc. may lower host resistance and result in conditions conducive for microorganisms of normal flora to cause opportunistic infections. Occasionally, highly pathogenic microorganism may appear in the normal flora without causing ill effects
- The normal flora is not static, although a basic flora is constant. Normal flora is divided into two types:
 - Resident flora—constant flora
 - Transient flora—variable flora
- Transient flora may include both pathogens and nonpathogens
- Detailed knowledge of the normal flora is necessary to understand the interaction between man and his pathogen-laden environment, which is of great importance in diagnostic microbiology in the interpretation of results of culture report
- The normal flora of different sites of the body is described in Fig. 14.1

■ Enumerate the advantages and disadvantages of normal microbial flora.

Advantages of Normal Flora

- The relationship between the commensals and their host is a symbiotic type of association in which both are benefitted
- They prevent or interfere with colonization/invasion of the body by more pathogenic organisms. This exclusion may be by simple competition with the pathogen for nutrition or by producing inhibitory substances
- Bacteria serve as scavengers and help in the disposal of waste matter, e.g. bacteria normally present in gut degrade mucins, epithelial cells, carbohydrate fibres and seem to be responsible for the normal structure and function of the intestine

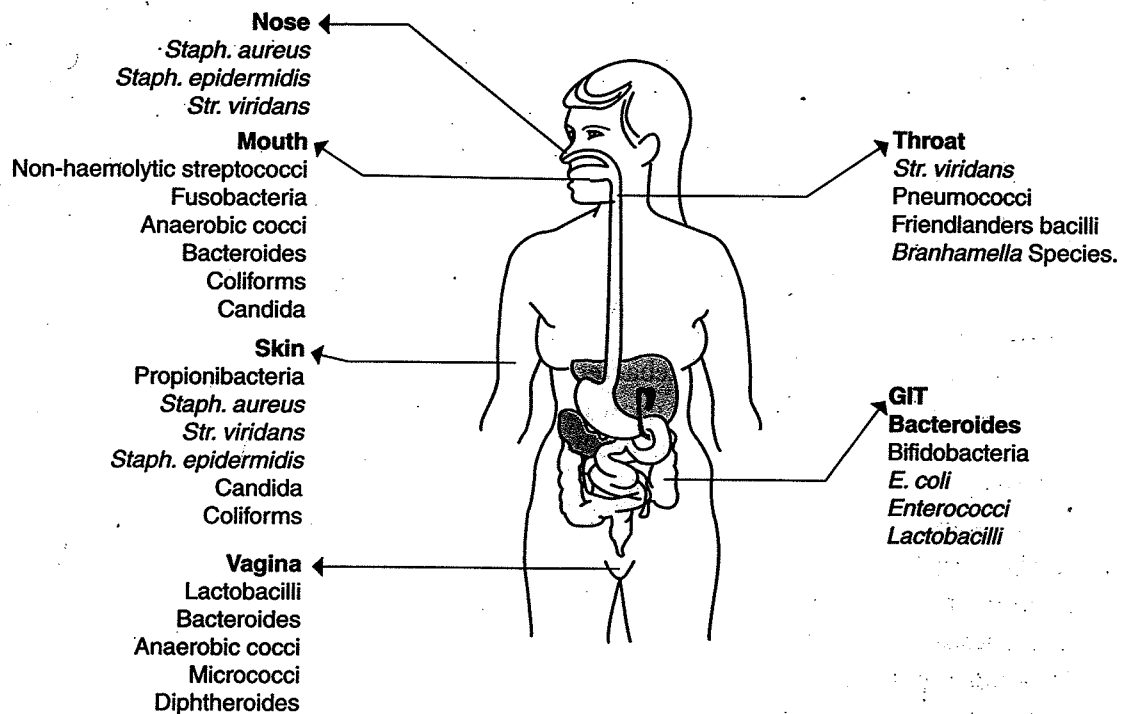


Fig. 14.1 Normal microbial flora of the body.

- The organisms normally present in the intestine produce the major B group vitamins, and also vitamin K and vitamin E. They also produce organic acids such as acetic acid, butyric acid, etc. and contribute in the nutrition of the host
- The organisms of normal flora increase the overall immune status of the host against pathogens having related or shared antigens

Disadvantages of Normal Flora

- The organisms of normal flora can become pathogenic and cause disease when resistance of the host is low
- They may act as pathogens in tissues other than their normal habitat, e.g. intestinal bacteria may cause urinary tract infections (UTI)
- They can aggravate infection, e.g. penicillinase-producing bacteria can aggravate infection by interfering with therapy
- They cause confusion in the diagnosis

Write a short note on normal flora of the skin.

- The skin is the outermost layer that constantly receives microorganisms from the air and objects with which it comes in contact, but the majority of them do not grow on skin because of the absence of favourable growth conditions and due to presence of bactericidal substances
- The normal flora of skin is of two types—transient and resident
- Transient microflora tends to occur more frequently on the skin than resident flora but it is unable to grow and usually die. The resident flora is rich— 10^2 – 10^4 organisms per cm^2 and comprises both aerobes and anaerobes

- The microorganisms, which are constantly present on the skin, are:
 - Gram-positive bacteria such as
 1. Anaerobic corynebacteria, e.g. *Propionibacterium acnes*
 2. Anaerobic cocci
 3. *Staphylococcus epidermidis*
 4. *Streptococcus viridans*
 5. *Str. faecalis*
 6. Micrococci
 7. Nonpathogenic *Bacillus* spp.
 8. Aerobic nonpathogenic corynebacteria
 - The Gram-negative bacteria that grow on skin are:
 1. *Escherichia coli*—inoculated on skin by faecal contamination
 2. *Proteus* spp.
 3. *Acinetobacter* spp.
 - Other organisms which found normally on the skin are:
 1. Nonpathogenic mycobacteria
 2. *Candida albicans*
 3. Other fungi, e.g. cryptococci and *Pityrosporum ovale*
 4. In 50% of normal adults *Staph. aureus* is present on the skin
 5. The pathogenic organisms such as *Salmonella typhi*, *Str. pyogenes*, etc. are quickly eliminated from skin because of bactericidal substances

■ Write a short note on normal flora of the conjunctiva.

- The conjunctival sac is generally free from organisms because of lysozyme and flushing action of tears
- The organisms are scanty and occasionally present
- The organisms present in conjunctiva are:
 - *Corynebacterium xerosis*
 - *Staph. epidermidis*
 - *Moraxella* spp.
 - Nonhaemolytic streptococci

■ Write a short note on normal flora of the mouth.

In spite of antibacterial activity of lysozyme and peroxidase in saliva, the mouth has a more complex and mixed flora. The abundant moisture and the presence of small food particles provide an ideal environment for bacterial growth. Both aerobes and anaerobes are present normally (Table 14.1).

Table 14.1 Normal flora of the mouth

Nonhaemolytic streptococci	Peptococci
<i>Neisseria</i> spp.	Peptostreptococci
Micrococci	<i>Bacteroides</i> spp.
<i>Bacillus</i> spp.	<i>Fusobacterium</i> spp.
Coliform bacteria	<i>Actinomyces</i> spp.
<i>Proteus</i> spp.	Nonpathogenic spirochaetes
<i>Mycoplasma</i> spp.	Lactobacilli
<i>Candida</i> spp.	<i>Veillonella</i> spp.
<i>Corynebacterium</i> spp.	

■ Write a short on normal flora of the upper and lower respiratory tract.

- The lower respiratory tract is normally sterile
- The upper respiratory tract is normally heavily colonized
- The organisms most frequently and most constantly present in nose are nonpathogenic corynebacteria
- The nose is also the natural home of *Staph. aureus*
- Other organisms, normally present, are:
 - *Staph. epidermidis*
 - *Str. viridans*
 - *Neisseria catarrhalis*
 - *Moraxella lacunata*
 - *Haemophilus* spp.

In throat

- *Str. viridans* and *N. catarrhalis* are commonly present
- Pneumococci and Friedlander's bacillus are also frequently seen
- The nasopharynx is a natural habitat of the common pathogens causing infections of the nose, throat, bronchi and lungs
- *Pseudomonas aeruginosa*, *E. coli*, and *Proteus* species may also be seen occasionally in normal individuals

■ Write a short note on normal flora of the gastrointestinal tract (GIT).

- The healthy stomach is practically sterile because of hydrochloric acid with pH 2, which is not suitable for microbial growth
- Duodenum is also fairly acidic and resembles stomach and lacks organisms
- From duodenum to ileum, pH gradually becomes less acidic and the number of organisms progressively increases from duodenum. In lower ileum about 10^5 – 10^7 organisms/gm of content are present
- In large intestine (caecum and colon), counts of 10^{10} – 10^{11} organisms/gm of content are present
- In an adult, the normal flora is mostly anaerobic (96–99%) and about 1–4% are aerobic bacteria
- The intestinal flora consists of both aerobes and anaerobes (Table 14.2)
- The gastrointestinal tract of the fetus *in utero* is generally sterile. It is colonized with organisms within 4–24 hours
- The intestinal flora of breastfed infants contains lactobacilli, but on substitution of solid food and diet there is predominance of Gram-negative bacilli

Table 14.2 Normal intestinal flora

Aerobes	Anaerobes
<i>E. coli</i>	<i>Bacteroides</i> spp.
<i>Str. faecalis</i>	<i>Bifidobacterium</i> spp.
<i>Proteus</i> spp.	Lactobacilli
<i>Enterobacter</i> spp.	Clostridia
<i>Pseudomonas aeruginosa</i>	Peptostreptococci
<i>Mycoplasma</i> spp.	<i>Eubacterium</i> spp.
<i>Candida albicans</i>	<i>Leptotrichia</i> spp.

- Faeces contain large number of bacteria, constituting 15–30% of the faecal mass. The majority of these bacteria seem to be dead
- The number of living bacteria in faeces is about 10^{10} organisms/gm and almost all (99.9%) are anaerobes

■ **Write a short note on normal flora of the genitourinary tract.**

- The female genital tract is heavily colonized than male because of its anatomy
- The normal vaginal secretions contain up to 10^8 bacteria/ml
- The organisms normally present are enumerated in Table 14.3
- Smegma bacillus (*Mycobacterium smegmatis*) is normally present in the secretion of both males and females. It may cause confusion in the diagnosis of renal tuberculosis, if the specimen of urine is contaminated by them
- During pregnancy, there is predominance of *Staph. epidermidis*

■ **Which microorganisms are commonly found into the blood and tissues?**

Commensal organisms from normal flora of the mouth, nasopharynx, intestinal tract and other sites may enter into the blood and tissues, but they are quickly eliminated by the defense mechanism.

Table 14.3 Normal flora of the genitourinary tract

Aerobes	Anaerobes
Micrococci	Lactobacilli (most predominant)
Diphtheroides	<i>Bacteroides</i> spp.
<i>E. coli</i>	<i>Bifidobacterium</i> spp.
Smegma bacilli	<i>Eubacterium</i> spp.
<i>Staph. epidermidis</i>	Peptococci
<i>Str. faecalis</i>	Peptostreptococci
Genital <i>Mycoplasma</i>	<i>Treponema</i> spp.
<i>Gardnerella vaginalis</i>	<i>Clostridium</i> spp.
<i>Corynebacterium</i> spp.	Anaerobic streptococci
<i>Candida</i> spp.	<i>Veillonella</i> spp.
<i>Neisseria</i> spp.	<i>Fusobacterium</i> spp.
<i>Listeria</i> spp.	
Alpha haemolytic streptococci	
Beta haemolytic streptococci	
Other streptococci	
<i>Klebsiella</i> spp.	

15

Chapter

Antimicrobial Susceptibility Testing

■ What is the importance of antimicrobial susceptibility testing?

Antimicrobial susceptibility testing *in vitro* is performed to determine

- The susceptibility of a given microorganism to known concentrations of the drug
- The potency of an antimicrobial agent
- The concentration of antimicrobial agent in body fluids or tissues

The test is routinely employed in clinical laboratory for determination of susceptibility of clinical isolates. As susceptibility varies from place to place and hospital to hospital and even some bacteria exhibit variations within the hospital, it is obligatory to test its susceptibility to institute rational antimicrobial therapy.

■ Name the tests generally performed for assessing antimicrobial susceptibility of clinical isolates.

Two types of antimicrobial susceptibility tests are:

1. Diffusion tests
2. Dilution tests

■ Discuss in detail 'Diffusion tests', methods of antimicrobial susceptibility testing.

Diffusion tests are most widely used to determine the susceptibility of clinical isolates to antimicrobial agents that are likely to be used in the treatment.

Principle

To allow the antimicrobial agent to diffuse through a solid medium so that the concentration is highest near the site of application of antimicrobial agent and decreases with distance.

- Many methods are available for implementation of diffusion tests. These include:
 1. Disc diffusion method
 2. Cup method
 3. Cylinder method

The most commonly used method is disc diffusion method.

Disc Diffusion Method

It is the most simple and easy method, hence most commonly used.

Principle

The method involves the addition of known amount of an antimicrobial agent to a filter paper disc measuring 6 mm in diameter. The placing of this disc on the agar surface previously

inoculated with bacterium to be tested will result in development of a zone of inhibition of growth around the disc.

Recommended Methods for Disc Diffusion Testing

The following methods are used:

1. Kirby-Bauer disc diffusion method
2. Stokes disc diffusion method
3. Epsilometer test

Kirby-Bauer Disc Diffusion Method

It is a simple and reliable method, especially applicable in routine clinical bacteriology.

Requirements

- * **Discs of antimicrobial agents**—these are discs of a standard filter paper charged with appropriate concentration of the antimicrobial agent. Two types of discs are used:
 - Low potency discs—for bacterial isolates from specimens other than urine
 - High potency discs—for bacterial isolates from urine
- * **Culture medium**—which supports the growth of most of the organisms. The following media are used:
 - Mueller-Hinton agar
 - Nutrient agar—free from inhibitory substances
 - Blood agar/chocolate agar—for *Haemophilus influenzae* and other fastidious bacteria, which do not grow on nutrient and Mueller-Hinton agar
- * **Inoculum** of test bacterium in a suitable broth medium (e.g. peptone water). For preparation of inoculum, 4 or 5 colonies of test organism are inoculated into a broth medium and incubated at 37°C until the turbidity (10^7 CFU/ml; colony forming unit per millilitre) of the culture matches with the recommended turbidity standard (0.5 of McFarland opacity standard tube)
- * **Control cultures**—Results of disc diffusion tests vary with a number of experimental conditions, e.g. pH, depth of medium, hydration, concentration of agar, conditions of incubations, etc., which are difficult to standardize. Hence, it is necessary to evaluate results by comparing with the results of control test of standard organisms of known susceptibility. The controls used are:
 - *Staph. aureus*—ATCC 25923
 - *E. coli*—ATCC 25922
 - *Pseudomonas aeruginosa*—ATCC 27853

Procedure

- * A standardized inoculum is inoculated with the help of a sterile cotton swab on the surface of the agar plate and the plate is allowed to dry for 3–5 minutes
- * Discs of antimicrobial agents are applied to the surface of agar plate, either by a mechanical dispenser or by hand using sterile forceps. The discs are placed firmly
- * On a plate of 100 mm, seven discs—one in centre and six in the periphery are placed
- * Control strains of *Staph. aureus*, *E. coli*, *P. aeruginosa*, etc. should also be tested each time
- * The plates are kept in refrigerator at 4°C for 30 minutes for diffusion and are then incubated at 37°C for 16–18 hours (Fig. 15.1)

Results

The diameter of the zones of growth inhibition around each disc is measured with the help of callipers or by viewing the plate against a ruler or ruled screen. The zones are

compared with zones of inhibition of standard control strain and results are interpreted as:

- **Sensitive**—when zone diameter of test organism is greater than, equal to or not more than 4 mm less than that of control strain
- **Intermediate sensitive**—if its zone diameter is at least 12 mm but reduced by more than 4 mm as compared to control strain
- **Resistant**—if it shows no zone of inhibition of growth or if the zone diameter is not more than 10 mm

Alternatively, the results are interpreted based on the standard interpretation chart of zone diameters.

Uses

To determine the susceptibility of clinical isolates to antimicrobial agents.

Stokes Disc Diffusion Method

This method is similar to Kirby-Bauer disc diffusion method. The only change employed in this method is that the control culture and test culture are grown on the same plate to compare the zones of inhibition produced in two cultures by the same disc.

Procedure

The test bacterium is inoculated on middle third of culture plate and control culture on upper and lower thirds of the plate carefully leaving an uninoculated strip of about 5 mm between the test and control areas, which are used for placing of discs. After inoculation, discs are placed (maximum of six), three on each side between the control and the test inocula.

The plates are kept in refrigerator for diffusion and then incubated at 37°C for 16–18 hours (Fig. 15.2).

Result

The results are interpreted by measuring and comparing the zones of inhibition of control and test bacterium as:

- **Sensitive**—if the zone of inhibition by test bacterium is equal to or larger or not less than 3 mm than that of control strain
- **Intermediate sensitive**—if the zone diameter of the test bacterium is at least 2 mm and the difference between the zone of test and control strain is 3 mm
- **Resistant**—if the zone diameter of test bacterium is less than 2 mm

Uses

To determine the susceptibility of clinical isolates to antimicrobial agents.

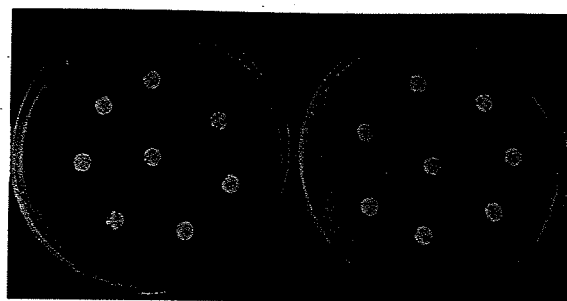


Fig. 15.1 Kirby-Bauer disc diffusion method.

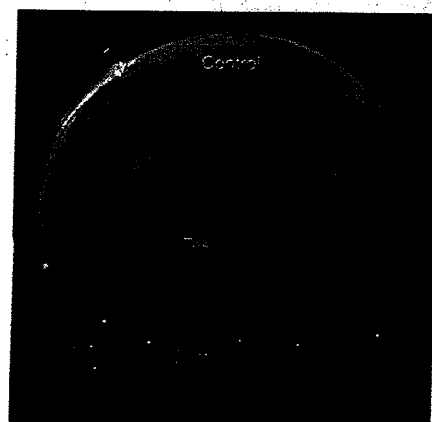


Fig. 15.2 Stokes disc diffusion method.

■ Epsilometer Test (E Test)

This method is a modification of agar diffusion sensitivity test used to detect minimum inhibitory concentration (MIC) of antibiotic.

Procedure

The test bacterium is inoculated and an absorbent strip with a known gradient of antibiotic concentration along with its length is placed on agar. The plates are kept in refrigerator for diffusion and then incubated at 37°C for 16–18 hours (Fig. 15.3). The antibiotic diffuses into the medium and inhibits the growth.

Result

The lowest concentration of the gradient which inhibits the growth of the organism is taken as MIC.

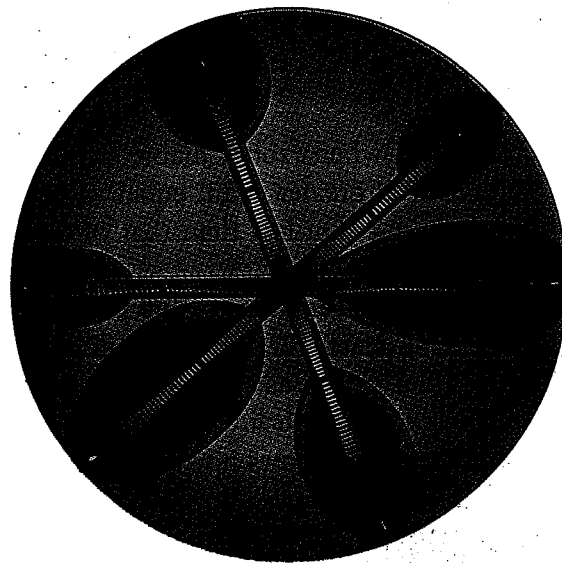


Fig. 15.3 Epsilometer or E test.

■ Name the automated AST systems.

Automated AST systems used for assessing antimicrobial susceptibility of clinical isolates are:

- Microscan walk away systems
- Sensititre ARIS
- Vitek system

■ Write a short note on (a) Broth dilution test and (b) Agar dilution test.

(a) Broth Dilution Test

Procedure

- Graded amounts of antimicrobial agents are incorporated into Mueller–Hinton broth in test tubes
- These media are then inoculated with test bacterium
- Control strain with known sensitivity is also inoculated in a separate set
- Incubated at 37°C for 16–18 hours

Results

- The end point is taken as minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC)
- Minimum inhibitory concentration—is the amount of antimicrobial agent required to inhibit growth
- Minimum bactericidal concentration—is the amount of antimicrobial agent required to kill the bacteria
- The MIC is detected by noting the lowest concentration of the antimicrobial agent showing no visible growth
- The MBC is detected by subculture from each tube showing no growth on a suitable medium without any antimicrobial agent. A loopful from each tube is inoculated over different sector or plate and plates are incubated at 37°C for 16–18 hours and the tube containing the lowest concentration of the antimicrobial agent that fails show growth on subculture is taken as MBC (Fig. 15.4)

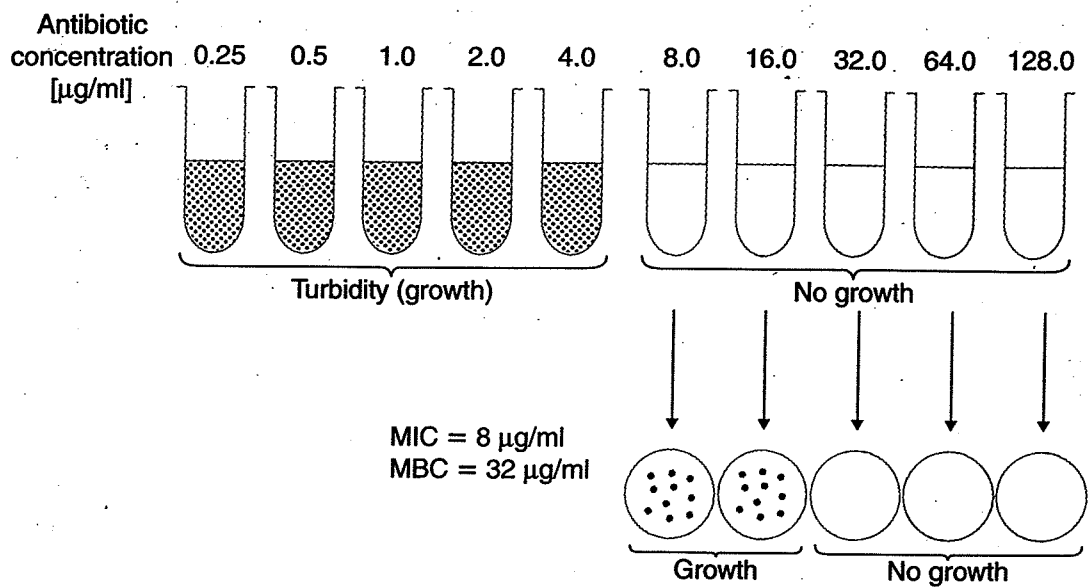


Fig. 15.4 Broth dilution test for MIC and MBC determination.

Uses

1. To regulate the therapeutic dose accurately, e.g. in the treatment of bacterial endocarditis
2. To study antimicrobial sensitivities of slow growing bacteria such as tubercle bacilli
3. To demonstrate small degree of resistance

(b) Agar Dilution Test

Procedure

- Serial dilutions of antimicrobial agents are prepared
- 1.5 ml of each dilution is added to 13.5 ml of melted agar suspension and poured into plates
- In this way, plates with different concentrations are prepared
- Many strains can be inoculated on each plate. Each strain under test is inoculated on agar plates containing different known concentrations of same antimicrobial agent
- A control plate without antimicrobial agent is also inoculated
- The plates are incubated at 37°C for 16–20 hours
- After incubation results are recorded

Results

The lowest concentration of antimicrobial agent that allows no more than one or two CFU or only a slight haze to grow is taken as MIC.

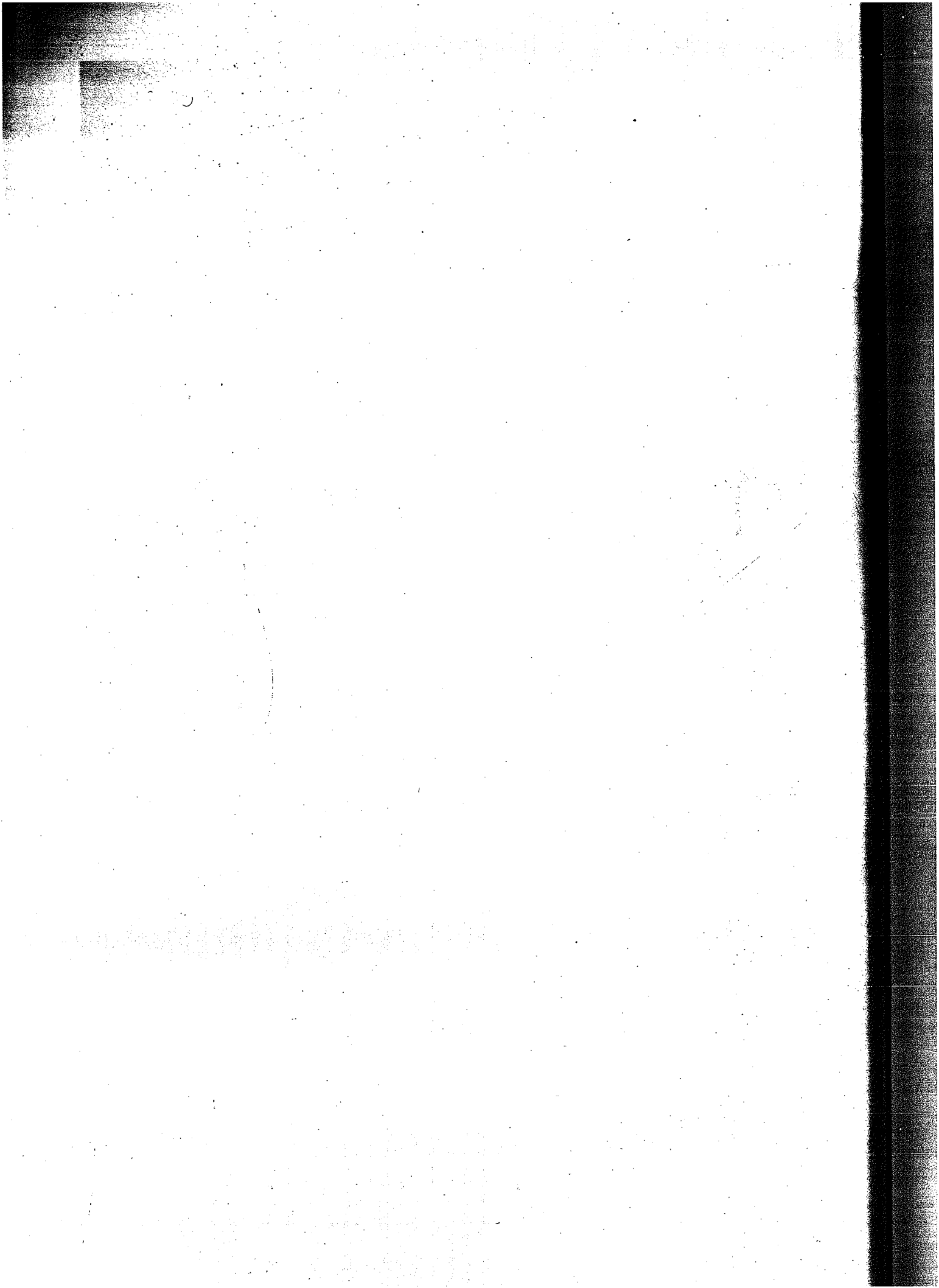
Uses

1. To study antimicrobial sensitivities of large number of isolates at a time
2. To determine the MICs of a large number of isolates

UNIT

III

Immunology



16

Chapter

Immunity

■ Define immunity.

Immunity is defined as the state of resistance or insusceptibility exhibited by the host towards injury caused by microorganisms or their products. It is the collective effort of cells, tissues and various molecules of the immune system to recognize and defend against infectious diseases.

■ Draw a flowchart to represent the different types of immunity.

Immunity can be natural (innate or nonadaptive) or acquired (adaptive). Flowchart 16.1 shows the features of both types of immunity.

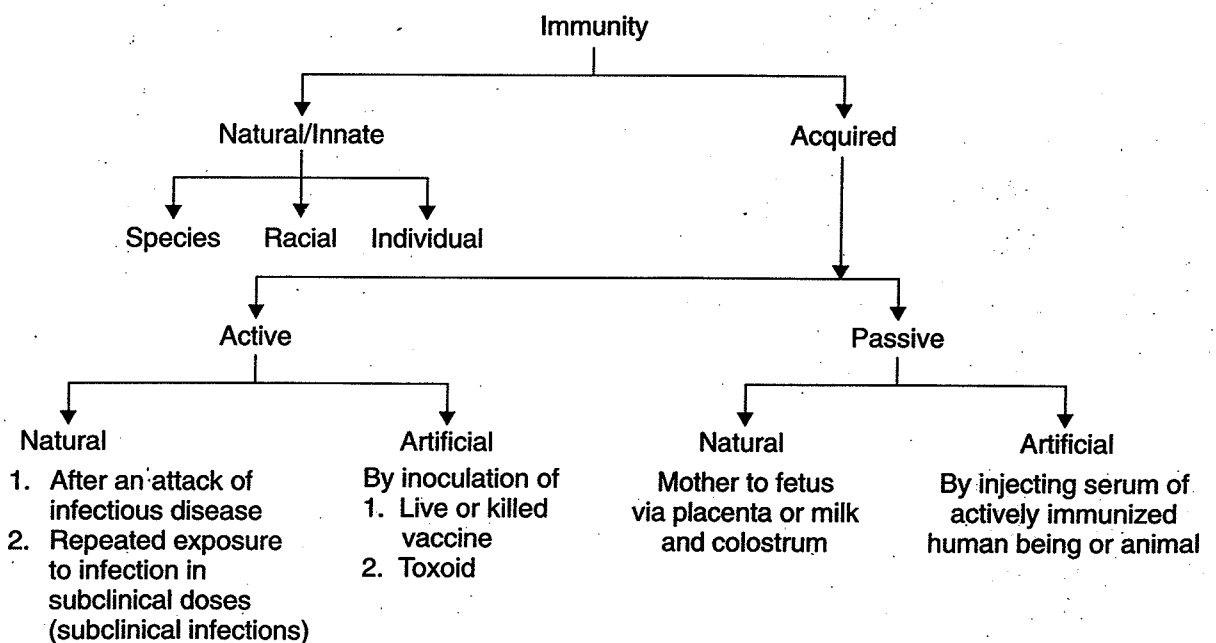
■ What is natural or innate immunity? Describe different types of natural immunity.

Natural or innate immunity is an inherited resistance to infections, which an individual possesses by virtue of his genetic and constitutional make-up. It is an intrinsic mechanism to defend against microbial infections, nonspecifically directed against any microorganism hence it is nonspecific defense mechanism of the body. It is also known as **constitutive immunity** or **native immunity**.

Types of Natural or Innate Immunity - three types (Flowchart 16.1)

Species Immunity

- This refers to immunity in the members of a particular species, e.g. many human pathogens do not infect animals and animals are naturally immune to many human pathogens, such as *Salmonella typhi*, *Vibrio cholerae*, gonococci and *Treponema pallidum*.



Flowchart 16.1 Types of immunity.

- Human beings suffer from mumps but dogs and cats do not
- Anthrax bacillus may infect mammals but not birds
- Amphibians are naturally immune to diphtheria and tetanus

This is because of species immunity. These differences may be due to physiological and biochemical differences between tissues of different species

Racial Immunity

Various races within the same species show marked difference in the degree of resistance to infections, e.g.

- Algerian race of sheep is resistant to anthrax while ordinary domestic sheep is susceptible to it
- 'Blacks' appear to be more susceptible to tuberculosis than 'whites'

These racial differences may be due to diet, habits, environmental conditions, lifestyle, economic status and genetic basis.

Individual Immunity

Resistance to infection varies from individual to individual. Different individuals of the same species may show marked difference in their immunity. This is evident during an outbreak of an infectious disease in which

- Some individuals would entirely escape from it
- Some individuals will develop subclinical infections
- Some individuals might develop the disease

Also, the severity of disease may differ in different individuals. This is due to individual immunity.

■ How does nonspecific innate immunity operate?

Innate immunity operates nonspecifically in the following two ways:

1. By preventing entry of microorganisms—it is the first line of defense (the external defense system)
2. By interacting with infectious agent through activation of tissue factors (cellular and humoral factors) when the infective agent penetrates the body—it is the second line of defense (the internal defense system)

■ Describe the external defense system of the body.

The external defense system includes:

Epithelial Barriers

In order to produce disease, microorganisms must enter into the body. The simplest way to avoid infection is to prevent the entry of microorganisms. The intact skin and mucous membrane play an important role in preventing entry of microorganisms.

Role of Skin

- The skin consists of keratin, which is indigestible by most of the microbes. Thus, it is impermeable to most of the microbes as long as it is intact
- Most bacteria fail to survive on skin for a long time because of the inhibitory effect of saturated and unsaturated fatty acids in sweat and sebum
- Sweat also contains high concentration of salts, which are inhibitory to bacteria and fungi
- Acidic pH (5.2–5.9) of the skin prevents growth of bacteria

Role of Mucous Membrane

- Mucous membrane secretes mucus, which is a protective barrier
- Ciliary movements, coughing and sneezing, remove microbes trapped within the adhesive mucus
- Mucous secretions of the respiratory, alimentary and genitourinary tract contains a bactericidal substance known as lysozyme, which kills bacteria by acting on cell wall mucopeptide

Role of Body Secretions

- Body secretes various fluids, which play important role in defense mechanism
 - Skin secretions—sweat and sebum contain bactericidal substances
 - Tears contain lysozyme, which is bactericidal, also the flushing action of tears makes conjunctiva free from microbes and dust particles
 - Mucopolysaccharides in saliva inactivate bacteria and viruses
 - Gastric juice contains hydrochloric acid (HCl), which destroys bacteria and keeps stomach free from microbes
 - The flushing action of urine eliminates bacteria from urethra
 - Semen is believed to contain antibacterial substances, e.g. spermine

■ Describe in detail the internal defense system of the body.

The internal defense system includes:

Tissue Factors

When the infective agent penetrates the body by passing the barriers of external defense, the tissue factors come into operation. There are two types of tissue factors:

1. Humoral Factors

Apart from specific antibodies, a variety of substances possessing antimicrobial activity are present in blood and tissue fluids. These include:

- **Lysozyme:** It is a heat labile, low molecular weight protein present in polymorphonuclear leucocytes and most of the tissue fluids except urine and cerebrospinal fluid. It is a mucolytic enzyme that acts by hydrolyzing the glycosidic linkage in the mucopeptide of the bacterial cell wall
- **Complement:** It is a nonspecific, heat labile protein normally present in serum that has antimicrobial activity. It plays an important role against pathogens invading blood and tissues
- **Properdin:** It is a complement-like substance normally present in serum. With the help of complement components and Mg^{2+} ions, it exerts antibacterial and antiviral activity, particularly active against Gram-negative bacteria especially shigellae
- **Interferon:** It is a nonspecific antiviral agent that interferes with intracellular viral replication. It also increases the activity of nonspecific killer cells
- **Phagocytin:** It is a heat stable protein derived from polymorphs. It is bactericidal in nature
- **Other antimicrobial substances:** Some substances such as beta lysin in serum, plakins derived from platelets, leukins derived from leucocytes, etc. are also active against infectious agents

2. Cellular Factors

Natural defense against microbes invading blood and tissues is mediated by phagocytic cells that engulf and digest them. Phagocytosis is the most important means of defense against microbes. There are two types of phagocytic cells:

1. **Microphages:** These are polymorphonuclear leucocytes or granulocytes in the blood. These include neutrophils mainly and eosinophils to a lesser extent
2. **Macrophages:** These are mononuclear cells—monocytes in blood and tissues

Their function is to recognize microbes and to initiate engulfment and activate the lytic enzymatic action to kill the ingested microbes.

In addition to phagocytic cells **natural killer cells** (a type of lymphocytes) that act on intracellular microbes by killing infected cells and by secreting cytotoxic proteins. These cells are particularly active against viral and tumour antigens.

Inflammation

Population of mast cells, which are extremely sensitive to injury, are distributed throughout the connective tissue, particularly in skin, lungs and intestine. Tissue injury or irritation initiated by the entry of microorganisms, toxins, or other factors (heat, trauma, etc.) cause degranulation of mast cells releasing various mediators that are responsible for an acute inflammatory response, which is an important nonspecific defense mechanism.

An inflammation occurs as a result of

1. Increased blood flow
2. Aggregation of macrophages and microphages by chemotactic mechanism

Role in defense

- The attracted phagocytic cells engulf and destroy the pathogens
 - The outpouring of plasma helps to dilute the toxic products
 - Acute phase protein reactants including fibrinogen, transferrin, C-reactive protein, complement components and others formed during inflammatory process serve as nonspecific resistance factors and may also participate in the tissue repair process. Thus, inflammatory response helps
 - To restrict infection
 - To limit tissue damage
 - To initiate tissue repair

Fever

Fever is a protective defense mechanism of the body. Increase in body temperature helps in the following ways:

- It increases circulation of blood and flushing of tissue that help to eliminate toxin through urine and sweat
- Increased body temperature may be harmful to invading microbes and in some instances may destroy the pathogens
- It stimulates the production of interferon that helps in recovery of viral infections

■ What is acquired immunity? Give the name of two types of acquired immunity.

- This refers to acquired resistance to infections which an individual acquires during the course of his/her life. It is immunity specific for a particular disease, hence it is also known as **specific immunity**
- It is of two types:
 1. Active immunity—induced by infection or vaccination
 2. Passive immunity—conferred by transfer of antibodies or lymphocytes from an actively immunized individual

■ What is active immunity? Briefly describe naturally acquired and artificially acquired active immunity.

- It is the resistance acquired by an individual in response to the microbes or their products (antigenic stimulus). The entry of antigen results in activation of immunocompetent cells

producing antibodies (humoral/antibody-mediated immunity) or sensitized T-cells (cell-mediated immunity)

- When exposed to the antigens of a microbe, an individual mounts an active type of immune response to eradicate the infection and becomes resistant to further infection by the same microbe.
- Active immunity requires a considerable time for its development (weeks or months known as latent period) but once developed, it persists for long duration and may last for years. Also it is associated with immunological memory. The memory cells are produced during the first contact (primary response) with antigen and retain the memory for long periods and give rapid and vigorous response (secondary response) when the same antigen enters again
- Active immunity is of two types:
 1. Naturally acquired active immunity
 2. Artificially acquired active immunity

Naturally Acquired Active Immunity

- Active immunity that develops naturally as a result of natural contact with a microbe or its product is known as naturally acquired active immunity
- This contact may result in a major invasion with clinical disease or a minor invasion without clinical disease (inapparent or subclinical infection)
- Following contact with antigen as a result of infection, the patient, in most cases, will be immune to further infection by the same pathogen for a period, which is different in different diseases. In diseases like influenza, common cold, gonorrhoea, the immunity lasts for short duration, whereas in other infections like diphtheria, small pox, measles, yellow fever, etc. it lasts for long duration and may persist for life
- In general, the immunity following viral infections lasts longer than immunity following bacterial infections
- In syphilis and malaria, a special type of immunity known as **premunition** or infection immunity is seen, i.e. immunity to reinfection persists as long as the original infection remains active. Once the infection is cured and the organisms are eliminated from the body, the patient again becomes susceptible to infection by *Treponemapallidum* and malarial parasites

Artificially Acquired Active Immunity

- Active immunity, which an individual acquires as a result of artificial inoculation of microbes or their products, i.e. immunization with microbes or their products. For immunization various immunizing agents such as vaccines or microbial products obtained from microorganisms, e.g. toxoid, extracted cellular fractions, etc. are used
- Vaccines are preparations containing live or killed organisms
- **Live vaccines**—are preparations containing live microorganisms with reduced virulence, e.g.:
 - Bacterial vaccines – BCG vaccine for tuberculosis and typhoral for enteric fever
 - Viral vaccines – Oral polio vaccine for polio and MMR for mumps, measles, rubella
- Live vaccines are prepared by using attenuated strains of microorganisms
- Live vaccines initiate infection without causing disease
- The level of immunity induced is same as natural infection and immunity lasts for several years
- Booster doses are generally not required but may be required sometimes
- Can be administered by the route of natural infection and parenterally
- **Killed vaccines**—are preparations containing killed or inactivated microorganisms. They are prepared by inactivation of microorganisms by heat, phenol, formalin, etc., e.g.
 - Bacterial vaccines – TAB for enteric fever and cholera vaccines for cholera
 - Viral vaccines – Salk vaccine for polio and rabies vaccines for rabies
- Killed vaccines do not initiate infection and there is no multiplication of microorganism

- The level of immunity induced is less as compared to natural infection and live vaccine, and also it lasts for short duration, hence booster doses are necessary
- Cannot be administered orally. They are effective by parenteral route
- **Toxoid**—Certain microorganisms produce exotoxins, e.g. diphtheria and tetanus bacilli. These toxins can be detoxified and used for immunization. The detoxified toxin is known as toxoid, which is nontoxic but antigenic. The toxoids are prepared by treating toxin with formalin or heat. Toxoid when injected, produces antitoxin, which specifically reacts with toxin and neutralizes it, e.g. TT (tetanus toxoid) for tetanus, DT (diphtheria toxoid) for diphtheria

■ Write a short note on passive immunity.

The immunity, which is acquired by transfer of readymade antibodies against microbes or their products in another host, is known as passive immunity. Here, the immune system does not take any active part in the development of immunity. As compared to active immunity, it is rapidly established and immediate protection is offered, which is necessary in certain clinical situations like diphtheria, tetanus, snake bite, etc.

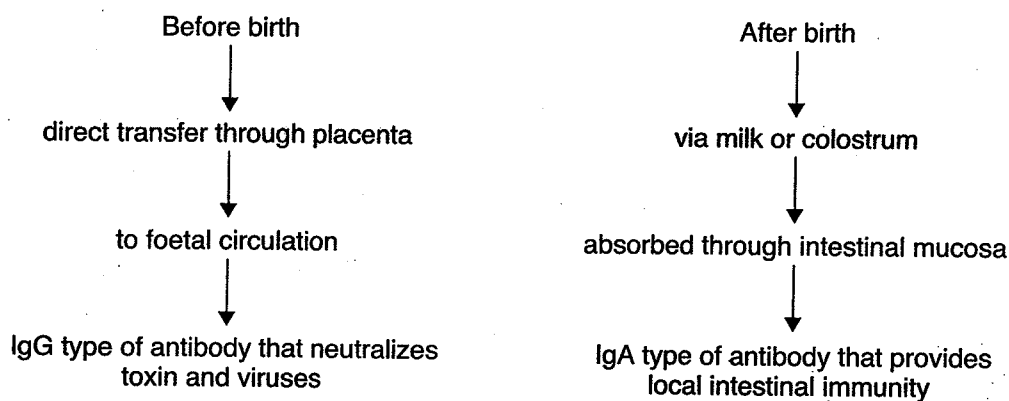
Passive immunity is of two types:

1. Naturally acquired passive immunity
2. Artificially acquired passive immunity

Naturally Acquired Passive Immunity

In newborn babies, generally there is no acquired active immunity because of immature immune system but they are resistant to number of infections such as measles, chicken pox, diphtheria, etc. This resistance is due to transfer of readymade antibodies from mother to fetus. It lasts for three to four months.

Transfer of antibody from mother to fetus occurs in two ways (Flowchart 16.2).



Flowchart 16.2 Pathway of transfer of antibodies from mother to fetus.

Artificially Acquired Passive Immunity

- Passive immunity can be acquired artificially by injecting antibodies produced in some other human or animal. This immunity lasts for short duration, e.g. the half-life of diphtheria antitoxin is 7 days. This procedure is used for
 - Treatment and also for prophylaxis and is particularly useful in clinical emergencies, where immediate and temporary protection is needed, e.g. snake bite, tetanus, diphtheria, etc.
 - Suppression of active immunity when it is injurious as in Rh-negative women with Rh-positive babies

- For passive immunization, the hyperimmune serum prepared in horse (most common), sheep, goat, rabbit, guinea pigs or human beings is used. The serum prepared may be antitoxic, antibacterial, or antiviral in nature and termed antiserum or antitoxin
- The antiserum is prepared by injecting specific antigen that leads to formation of specific antibody, e.g. rabies antiserum
- Antitoxin is prepared by injecting toxin or toxoid, e.g. anti-tetanus serum (ATS), anti-diphtheria serum (ADS), anti-snake venom (ASV), anti-gas gangrene serum (AGS), etc.
- Antisera and antitoxins are used only in clinical situations where no other alternative is available because of the possible risk of hypersensitivity reactions such as
 - Development of anaphylactic shock
 - Serum sickness

Occasionally, specific human immunoglobulins prepared from serum or plasma of convalescent or specifically immunized individuals (individuals who have been recently recovered or those who have been hyperimmunized with a specific antigen) are used for passive immunization. These are used in hepatitis B virus, rabies virus and other infections. Use of this preparation minimizes the risk of hypersensitivity reactions.

■ Differentiate between active and passive immunity.

The features distinguishing active and passive immunity are presented in Table 16.1.

Table 16.1 Differences between active and passive immunity

Active immunity	Passive immunity
1. Produced actively with the involvement of immune system	Received passively (readymade antibodies) without involvement of host's immune system
2. Induced by infection or by contact with antigen, e.g. vaccines, toxoid	Induced by injecting serum containing readymade antibodies prepared in animals or human beings
3. Takes time to develop antibodies or T-cells	Immediately established
4. Immunity lasts for long duration and is effective.	Lasts for short duration (10–14 days) and is less effective
5. Negative phase may occur after injection of antigen	No negative phase
6. Immunological memory present, booster doses more effective	No Immunological memory; subsequent administration of antibody less effective
7. Used for prophylaxis or treatment of sub-acute or chronic infections to increase resistance of the body	Used for treatment of acute infections for providing immediate and temporary protection. Also for prophylaxis

■ Briefly describe the types of immunity other than active and passive immunity.

Types of immunity other than active and passive immunity are:

1. Local Immunity

- Certain microorganisms infect particular groups of cells and selective tissues. Corresponding cells and tissues mediate immunity against such pathogens. This type of immunity mediated by corresponding cells and tissues is known as local immunity, e.g. in poliomyelitis, systemic immunity developed as a result of active immunization with killed vaccine (Salk vaccine) is able to neutralize viruses that enter the blood stream but are unable to prevent the local multiplication of viruses at the site of entry, i.e. intestinal mucosa. The local multiplication of viruses at the site of entry can be prevented by the local immunity developed as a result of infection or immunization with the live oral polio vaccine (Sabin vaccine).

- A special type of antibody IgA—plays an important role in local immunity. This IgA antibody, known as secretory IgA, is produced locally by plasma cells present on mucosal surfaces or in secretory glands. It is the main component present in various body secretions such as mucus of respiratory, intestinal, urinary and genital tracts, tears, saliva, milk, etc. and play a significant role in local immunity.

2. Herd Immunity

It is the overall level of immunity in the community and is important in the control of outbreak of infectious diseases.

- When herd immunity is low—an infectious disease spreads rapidly and may be severe in nature
- When herd immunity is high—an infectious disease spreads less rapidly and is of mild form
- It may be possible to eradicate communicable diseases like diphtheria, polio, etc. by developing high level of herd immunity by means of artificial active immunization

17

Chapter

Antigen (Ag)

■ Explain what an antigen is?

Antigen is defined as any substance, which when introduced parenterally (*para* means beyond or outside of and *enteron* means intestine), i.e. by routes other than gastrointestinal tract, into the living animal body, evokes specific immune response either by producing specific antibody or by producing specifically sensitized T-cells or both. The antibodies or T-cells formed in response to antigen specifically react against the antigen responsible for their production. Any foreign substance may act as an antigen, e.g.

- Dead or living microorganisms
- Vegetable proteins
- Egg albumin
- Serum
- Plant or animal tissue
- Bacterial toxins
- Red blood cells
- Snake venom
- Milk

■ Mention two attributes of antigenicity.

The two attributes of antigenicity are:

1. **Immunogenicity:** It is the capacity to provoke specific immune response
2. **Immunological reactivity:** It is the specific reaction of an antigen (Ag) with corresponding antibody or specifically sensitized T lymphocytes

■ Classify antigens on the basis of the response they are able to induce in the host.

Based on the ability of antigens to carry out the two functions (attributes), antigens (Ags) are classified into two types:

1. **Complete Ag (immunogen):** It is an Ag which is able to induce antibody formation and react with it specifically and in an observable manner. It is able to generate an immune response by itself and thus functions as antigen as well as immunogen.
2. **Hapten (incomplete Ag):** It is a substance which is unable to induce antibody synthesis by itself but can react specifically with antibody. Thus, hapten can function as an antigen but not as an immunogen. Haptens are incomplete Ags, which become complete Ags when they react with carrier molecule or schlepper. Haptens are generally low molecular weight lipids and carbohydrates, e.g. capsular polysaccharide of pneumococci, cardiolipin, polysaccharide 'C' of beta - haemolytic streptococci, drugs like penicillin, etc. while carrier molecules are proteins such as serum albumin or globulin or synthetic polypeptides. A hapten may be of the following two types:

1. Simple

- Simple chemical substances
- Monovalent
- Can react with antibody but unable to precipitate the reaction

2. Complex

- Relatively large molecules
- Polyvalent
- React with specific antibody and able to precipitate the reaction, e.g. capsular polysaccharide of pneumococci

■ What is an antigenic determinant? List its properties.

- Antigenic determinant is the smallest unit of antigenicity represented by a small area on the antigen molecule possessing a specific chemical structure and steric configuration, which determines the specific immune response and reacts specifically with antibody; it is also known as **epitope**. An antigen possesses several epitopes and each epitope induces specific antibody (Ab) formation
- The properties of epitopes are:
 - Size: 25 to 35 Å
 - Molecular weight: 400–1000
 - The determinant groups on
 - i. Protein Ag—penta or hexapeptide
 - ii. Polysaccharide Ag—hexasaccharide
- A determinant is roughly five amino acids in size
- The site on the Ab molecule, which combines with respective epitope is known as paratope

■ Describe the properties of antigens (requirements of immunogenicity/factors affecting immunogenicity).

For any substance to be antigenic, it should have the following properties:

1. Foreignness

The first and the most important factor for immunogenicity is the foreignness of antigen. As recognition of self and nonself is an essential function of immune system, it does not respond to self-Ags, hence substances that are foreign to the circulation are antigenic and are able to mount an immune response. Substances which are more foreign, are more powerful antigens, e.g. Ags from distant species are more antigenic than Ags from related species.

2. Size

The substance must have certain minimum size. Extremely small molecules such as amino acids and monosaccharides are nonantigenic. Large molecules such as haemocyanin having molecular weight 6.75 million are highly antigenic. In general, substances having molecular weight less than 10,000 are weakly antigenic or nonantigenic. The effective immunogens have molecular weight greater than 10,000. Substances having molecular weight lower than 10,000 may be rendered antigenic by adsorbing them on large inert particles like bentonite or kaolin.

3. Chemical Nature

- Antigens are proteins, polysaccharides, lipids or nucleic acids
- Protein Ags are more effective than polysaccharide Ags
- Lipids and nucleic acids are less antigenic and their antigenicity can be enhanced by coupling them with proteins

- Not all proteins are antigenic, e.g. gelatin, histones and protamines are nonantigenic because of absence of an aromatic radical such as tyrosine, which is must for antigenicity
- As a general rule
 - Antigenicity increases with structural complexity
 - The substances with diverse chemical and structural properties are the most powerful antigens
 - Presence of aromatic amino acid increases antigenicity

4. Susceptibility to Tissue Enzymes

It should be susceptible to tissue enzymes. After administration into the body, antigen is processed by phagocytic cells and intracellular enzymes into appropriate fragments containing epitopes. This processing of antigen is necessary to expose the hidden determinants of antigen, which is essential to initiate an immune response. Substances which are resistant to this processing are not antigenic.

■ Explain antigenic specificity. Also categorize specificity of natural tissue antigens of animals.

The reaction between antigen and antibody is highly specific. An antigen reacts with its corresponding antibody only. Antigenic specificity depends upon the specific active sites on the antigen molecules, i.e. antigenic determinants. Antigenic specificity is determined by

- Single chemical **groupings** and even by a single acid or basic group and spatial configuration of the determinant group, which makes *dextro*, *levo* and *meso* isomers antigenically different. Even the spatial distribution of the radicals into the antigenic molecule at the *ortho*, *meta* and *para* positions make it antigenically different
- Antigenic specificity is not absolute and **cross reactions** may occur between similar or related antigens due to **stereochemical similarities**
- The specificity of natural tissue antigens of animals may be considered under different categories. These are as follows:

Species Specificity

Tissues of all members in a species possess species-specific antigen. Some degree of cross-reaction may exist between antigens from related species. This immunological relationship is similar to phylogenetic relationships. These immunological relationships between species-specific antigens are useful in

- Tracing evolutionary relationships
- Identifying the species of the organism to which blood and seminal stains belong (forensic application)

Isospecificity

Isoantigens (alloantigens) are antigens found in some, but not all members of a species. A species may be grouped into different groups based on its specific isoantigens, e.g. human erythrocyte antigens; based on them individuals are classified into different blood groups—A, B and O. These antigens are genetically determined and are important in

- Blood transfusion
- Isoimmunization during pregnancy
- Providing valuable evidence in disputed paternity

Autospecificity

The autologous or self-antigens are generally not antigenic, but there are few exceptions, e.g. eye lens protein, thyroglobulin, etc. are anatomically confined to sites that prevent their contact with

immune system, hence they are not recognized as self-antigens. Also, sperms, which appear in latter life, are not recognized as self-antigens. When these antigens are released into circulation following injury to eye lens or damage to thyroid or testis and come in contact with immunocompetent cells, the result is formation of autoantibody producing autoimmune disease.

Organ Specificity

These are antigens confined to a particular organ or tissue. Such antigens present in an organ or tissue of different species are called organ-specific antigens. For example, brain tissue antigen of man, shares antigenicity with brain tissue antigen of sheep. The sharing of the antigens may result in the neuroparalytic complications in man following the antirabies vaccine containing partially denatured sheep brain tissue. The sheep brain tissue antigen induces immune response that produces damage to human brain tissue.

Heterogenetic or Heterophile Specificity

The same or closely related antigens present in different biological species, classes and kingdoms are known as heterogenetic or heterophile antigens. Antibodies to these closely related antigens produced by one species cross react with antigens of other species. The examples are:

- The Forssman antigen (Forssman 1911). It is a lipoprotein polysaccharide complex, which is widely distributed in man, animals, birds, plants and bacteria
- A heterophile antigen of Rickettsiae causing typhus fever is shared by certain strains of *Proteus* (OX 19, OX 2 and OX K). This forms the basis of Weil-Felix reaction in which *Proteus* strains are agglutinated by heterophile antibody in serum of a patient suffering from typhus fever
- Epstein Barr virus causing infectious mononucleosis shares antigenicity with sheep and Ox RBCs (Paul-Bunnell Test)
- In primary atypical pneumonia human 'O' RBCs are agglutinated by patient's serum (cold agglutination test)

18

Chapter

Immunoglobulins (Igs)—Antibodies (Abs)

■ Define antibodies.

Antibodies (immunoglobulins) are a group of glycoproteins present in the serum and tissue fluids of all mammals. They are synthesized in response to a foreign substance, i.e. antigen administered into the body with which they react specifically and in an observable manner.

■ Describe the characteristics of antibodies.

- Antibodies are globulins and are therefore, also known as immunoglobulins
- They contain sugar residues and hence are glycoproteins
- They constitute 20–25% of the total serum proteins
- When separated electrophoretically, most of them migrate in gamma region, hence they are also termed gamma globulins
- Most of them have molecular weight of 150,000–180,000 and sedimentation coefficient 7S–8S except for IgM. IgM has molecular weight of 900,000, sedimentation coefficient 19 S and termed M or macroglobulin
- They are thermolabile and denatured on heating at 70°C for 1 hour
- The aforementioned different terms for antibodies were in use earlier; currently, the generic term immunoglobulin (WHO, 1964) is internationally accepted for 'proteins' of animal origin endowed with antibody activity and other related proteins. The term immunoglobulin denotes chemical structure of protein while antibody refers to biological activity and function of proteins. Accordingly, immunoglobulins include abnormal plasma proteins known as abnormal immunoglobulins in addition to antibody globulins. Thus, all antibodies are immunoglobulins but all immunoglobulins may not be antibodies

■ With the help of suitable illustrations describe the structure of antibodies.

- The detailed structure of immunoglobulin was studied by Porter et al. (1962) by cleaving immunoglobulin molecule. They used rabbit IgG antibody to egg albumin
- The IgG can be digested by papain in the presence of cysteine into three fragments:
 - Two identical fragments having molecular weight 45,000 (sedimentation coefficient 3.5S), which are univalent and able to combine with antigen but unable to precipitate the reaction are called Fab (fragment antigen binding). It contains light chain and a part of heavy chain
 - The third fragment with molecular weight 55,000, containing a part of heavy chain only has no power to combine with antigen and is termed Fc (fragment crystallizable)
- When treated with another proteolytic enzyme—pepsin, a 5S fragment composed of two Fab fragments held together in a position is obtained. It is bivalent and precipitates with antigen. This fragment is known as F(ab)₂. The Fc portion is completely degraded by pepsin into smaller fragments (Fig. 18.1)
- Based on these findings, a basic four-chain model has been proposed by Porter et al. for immunoglobulin containing two distinct types of polypeptide chains—two heavy chains and two light chains linked together by disulphide (–S–S–) bonds (Fig. 18.2)

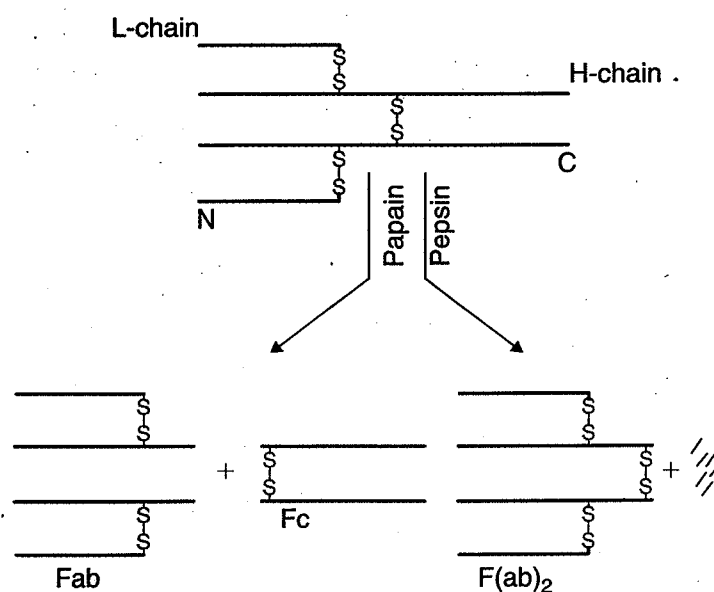


Fig. 18.1 Basic structure of Ig and fragments obtained by the action of papain and pepsin.

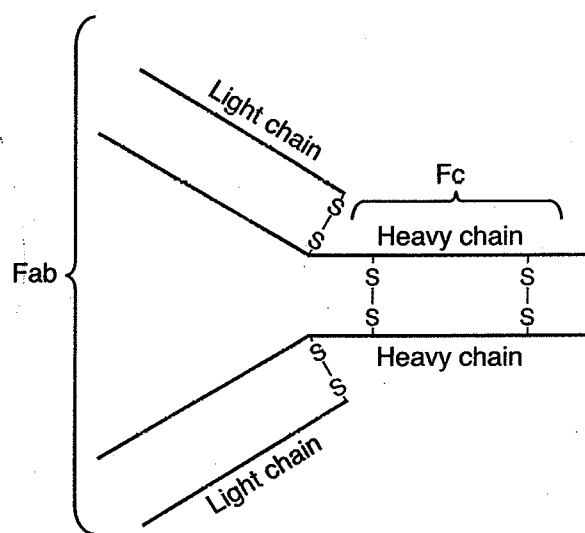


Fig. 18.2 A basic four-chain model.

- **Light chains** are smaller chains having molecular weight 20,000–25,000 and are attached to heavy chain by disulphide bonds. L chains are similar in all classes of immunoglobulins and occur in two groups kappa (κ) and lambda (λ). Antibody molecule may have either κ (60% of molecules) or λ (30% of molecules)
- **Heavy chains** are larger chains having molecular weight 50,000 and are linked together by disulphide bonds. H chains are structurally and antigenically different for each class of immunoglobulin and are designated by Greek letters (Table 18.1)
- The antibody molecule consists fragment antigen binding (Fab portion) responsible for binding with antigen. It is composed of both L and H chains
- It also consists of fragment crystallizable (Fc) portion, which is responsible for complement fixation, skin fixation, placental transfer, secretion into body fluids and binding to phagocytic and mast cells

Table 18.1 General properties of immunoglobulin molecules

Property	IgG	IgA (S IgA)	IgM	IgD	IgE
Type of heavy chain	Gamma	Alpha	Mu	Delta	Epsilon
Molecular weight	150,000	160,000 (385,000)	970,000	184,000	188,000
Sedimentation coefficient	7S	7S (11S)	19S	7S	8S
Half-life (days)	23	6–8	5	2.8	2.3
Carbohydrate content (%)	3	11	10	13	12
Valency	2	2 (4)	10	2	2
Normal serum concentration (mg/ml)	8–16	0.6–4.2	0.5–2	0–0.04	0.00003
Subclasses	Four— IgG1, IgG2, IgG3, IgG4	Two—IgA1, IgA2	Two—IgM1, IgM2	Two—IgD1, IgD2	—

Variable and Constant Region

Both H and L chains contain an amino terminal end; means there is a free amino group on the terminal amino acid. This is called variable region (V), which is different for each class and subclass of immunoglobulins because of variation in amino acid composition. They also contain carboxy terminal end, which means that there is a free carboxyl group on the terminal amino acid. This is called constant (C) region, whose composition is constant in all immunoglobulin molecules.

The antibody specificity depends upon the variability of the amino acid sequences at the variable region of the H and L chains. This antibody specificity is explained on the basis that the combining site of antibody molecule possesses a specific amino acid composition that is complementary to specific reactive area of the antigen molecule. Recently, hypervariable regions (paratopes) in variable portions of H (four) and L chains (three) have been identified. These hypervariable regions (hot spots) are involved in the formation of antigen binding site, which make actual contact with epitope.

Hinge Region

When antibody molecule is visualized under electron microscope, it appears as a Y-shaped structure whose arms can swing out to an angle of 180° through the papain and pepsin sensitive region called hinge region. Hinge region consists of a large number of proline residues.

Immunoglobulin Domains

The polypeptide chains do not exist as a linear sequence of amino acid molecules but are folded by disulphide bonds into globular regions known as domains. The H chain contains five domains—CH-1, CH-2, CH-3, CH-4 (domains in constant region) and VH (domain in variable region). The L chains contain two domains—CL (constant region) and VL (variable region). Each domain has a separate function (Fig. 18.3).

- The VL and VH domains are responsible for the formation of specific antigen binding site
- The CH-2 domain in IgG binds to C1q in classical complement pathway
- CH-3 domain mediates adherence to the surface of monocytes

■ **Mention the name of five classes of immunoglobulins. Describe the features and biological activities of each of them.**

- Human sera contain five different types of Igs—IgG, IgA, IgM, IgD and IgE in the decreasing order of concentration
- General properties of immunoglobulin molecules are summarized in Table 18.1

Immunoglobulin G (IgG)

- It is the major Ig in normal serum accounting for 70–80% of the total immunoglobulins
- It is equally distributed between the intravascular and extravascular compartments
- It is a monomer consisting two H and two L chains (Fig. 18.4)
- It is the major immunoglobulin synthesized during secondary response

Biological Activities

- In pregnant women, it has the ability to cross the placenta and reach the foetal circulation to provide a major line of defense (naturally acquired passive immunity) against infection in the newborn for the first few weeks
- It neutralizes viruses, activates complement and enhances phagocytosis
- In extravascular body spaces, it carries burden of toxin neutralization
- It is able to activate complement and thus helps to attract polymorphonuclear leucocytes (phagocytic cells) by chemotactic mechanism and stimulates ingestion and killing of microorganisms
- It reacts with the target cell with the help of Fab portion and mediates extracellular killing by K-cells bearing the specific receptor for Fc portion of IgG on target cell
- It participates in precipitation and complement fixation, and in allergic reactions, e.g. Arthus reaction and also in autoimmune diseases

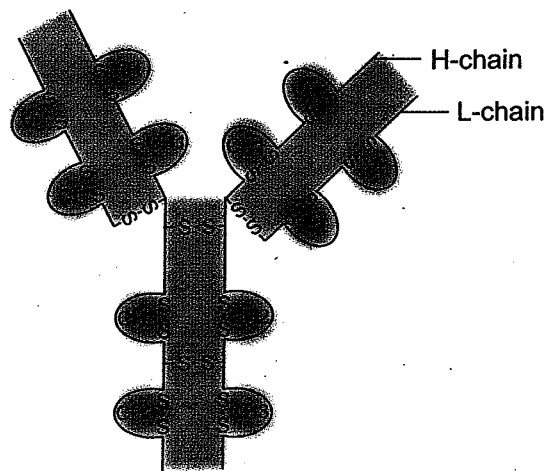


Fig. 18.3 Immunoglobulin domains.

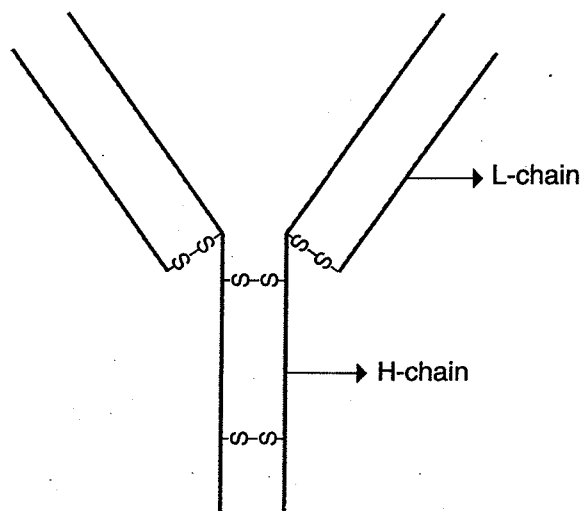


Fig. 18.4 Immunoglobulin G.

- When it is administered passively, it suppresses the homologous Ab synthesis by a feedback mechanism. This property is utilized in the isoimmunization of women during delivery by the administration of anti-Rh (D) IgG

IgG protects the body fluids

Immunoglobulin A (IgA)

- It is the second most abundant immunoglobulin constituting 10–13% of the total immunoglobulins
- It occurs in two forms—in human sera more than 80% of IgA occurs as monomer containing two H and two L chains but in most mammals it is polymeric
- Secretory IgA present in the seromucous secretions such as saliva, tears, nasal fluids, sweat, colostrum and secretions of the lungs, genitourinary and gastrointestinal tract is the dimer containing four H and four L chains. It possesses a cysteine rich polypeptide chain called J-chain (joining chain) of molecular weight 15,000 that joins two monomeric units of IgA
- Also possesses an additional structural unit—a glycine-rich polypeptide called the T (transport) or S (secretory piece or secretory component) having molecular weight 60,000, attached to the IgA molecule during transport across the cells. It appears to protect IgA from digestion by proteolytic enzymes (Fig. 18.5)

Biological Activities

- The IgA in secretions (secretory IgA) is synthesized locally by plasma cells situated near the mucosal or glandular epithelium and selectively concentrated in secretions and on mucous surfaces forming an Ab paste. It plays important role in local immunity against respiratory, intestinal and urogenital pathogens inhibiting their adherence to these mucosal surfaces and thereby preventing their entry into the body tissue
- They also activate complement by alternate pathway and promote phagocytosis and intracellular killing of microorganisms

IgA protects the body surface

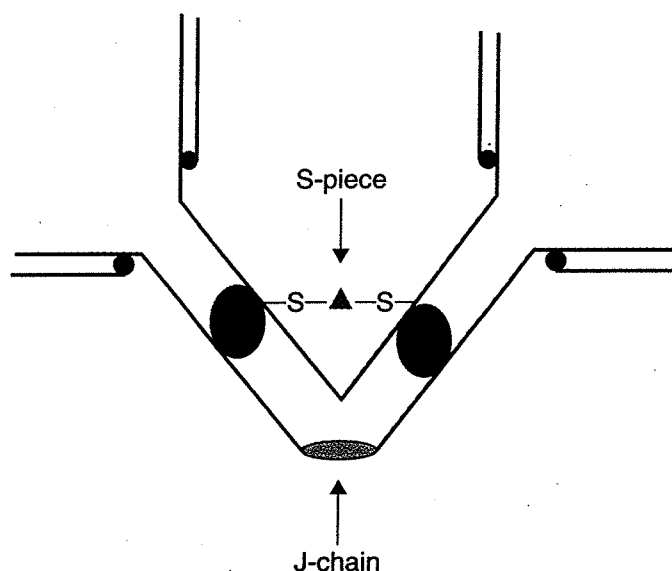


Fig. 18.5 Immunoglobulin A.

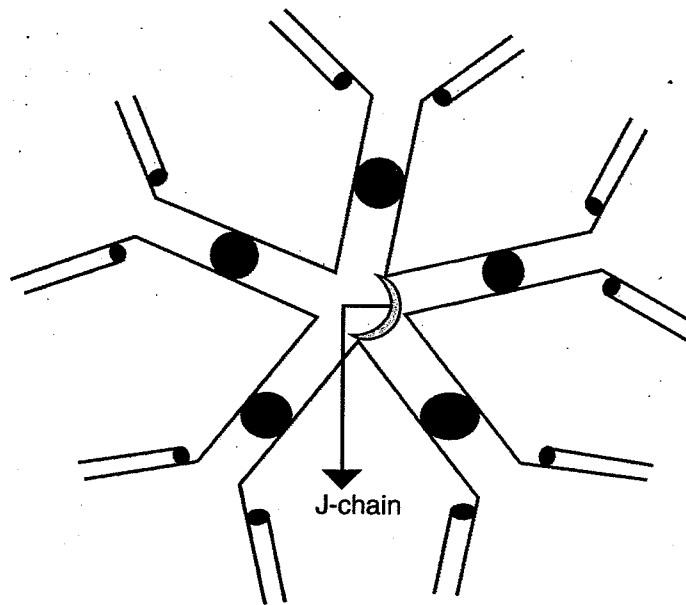


Fig. 18.6 Immunoglobulin M.

Immunoglobulin M (IgM)

- It is the first Ab formed in every response
- It constitutes 5–8% of serum immunoglobulins
- It is a pentamer containing 10 H and 10 L chains—five subunits of monomer joined together by J-chain (joining chain) (Fig. 18.6)
- It is susceptible to mercaptoethanol—serum treatment with mercaptoethanol selectively destroys IgM

Biological Activities

- It activates complement, neutralizes toxins and viruses
- It is most efficient in agglutination, complement fixation and cytolytic reaction, and also in immune haemolysis, opsonization and bactericidal action
- Most of the IgM (80%) is intravascular in distribution (confined to blood stream), hence it offers protection against bacteraemia and septicaemia
- As it is not transported across the placenta, its detection in fetus or newborn indicates intra-uterine infection, which is useful in the diagnosis of congenital syphilis, rubella, toxoplasmosis and HIV infection
- It fixes complement by classical pathway
- As it is a short-lived immunoglobulin that disappears rapidly, its demonstration in serum indicates recent infection
- Monomeric IgM appears on the surface of unstimulated B lymphocytes and acts as recognition receptor for antigens

IgM protects the bloodstream

Immunoglobulin D (IgD)

- It occurs in low concentration—accounts for less than 1% of the total immunoglobulins
- It is structurally similar to IgG
- It is a monomer containing two H and two L chains

Biological Activities

- It may function as antigen receptor on the surface of B lymphocytes for recognition of antigens and for the control of activation and proliferation of lymphocyte to produce antibodies

Immunoglobulin E (IgE)

- It occurs in very low concentration but the level is greatly elevated in atopic conditions such as asthma, hay fever and eczema
- It is structurally similar to IgG—monomer contains two H and two L chains
- It is susceptible to mercaptoethanol

Biological Activities

- It is extravascular in distribution and has affinity for the surface tissue cells, particularly mast cells and basophils. It causes degranulation of these cells, releasing pharmacologically active substances. Thus, it is responsible for Type-I hypersensitivity reaction. It also plays an important role in immunity against helminthic parasites

IgE—Mediates reaginic hypersensitivity

■ **What are abnormal immunoglobulins? Give three examples of diseases associated with production of abnormal immunoglobulins.**

Abnormal Immunoglobulins

- These are proteins, structurally similar to antibodies, present in serum in many pathological processes and even sometimes in healthy individuals. The abnormal immunoglobulins were first discovered by Bence Jones (1847) and are called Bence Jones proteins. These are typically seen in multiple myeloma and have characteristic property of coagulating when heated at 60°C but redissolving at 80°C. These are the light chains of immunoglobulins and may occur as *Kappa* or *Lambda* chains in the urine of about 50% patients of myeloma. In any one particular patient, the light chain is either *Kappa* or *Lambda* only. These are formed because of unchecked proliferation of one clone of plasma cells in myeloma that results into excessive production of the particular immunoglobulin and, therefore, such immunoglobulins are called monoclonal. Myeloma may affect plasma cells synthesizing any of the five classes of immunoglobulins—about 50–60% cases suffering from myeloma are IgG, 20% IgA, 10% IgM, 1–2% of IgD and myeloma with IgE is extremely rare.

Diseases Associated with Production of Abnormal Ig**Waldenstrom's Macroglobulinaemia**

This is myeloma-involving IgM-producing plasma cells. In this condition, there is excessive production of the respective myeloma proteins (M-proteins) and their light chains.

Heavy Chain Disease

This is a rare condition in which there is overproduction of the Fc portion of immunoglobulin heavy chains, which are excreted in urine of patients. This occurs in association with lymphoma.

Cryoglobulinaemia

It is a condition in which there is formation of gel or precipitate on cooling the serum. This precipitate redissolves on warming. It is often associated with macroglobulinaemia, myelomas and autoimmune diseases such as systemic lupus erythematosus (SLE). Most cryoglobulins are made of IgG, IgM or mixture of these two.

19

Chapter

Antigen–Antibody Reactions

■ **Explain antigen–antibody reaction. Mention the importance of these reactions.**

Antigen–Antibody Reaction

When specific antigen and antibody molecules are mixed together in solution, an antigen reacts with its antibody forming a complex. This complex formation in an observable manner due to combination of specific Ag with specific Ab is called Ag–Ab reaction.

Importance of Ag–Ab Reactions

- These reactions play important role in Ab-mediated immunity against infectious diseases and tissue injury in hypersensitivity and autoimmune diseases *in vivo* (in the body)
- In the laboratory, Ag–Ab reactions are useful in
 - Diagnosis of infectious and noninfectious diseases
 - Epidemiological surveys

In general, these reactions can be used for the detection and quantitation of either antigens or antibodies

- The Ag–Ab reactions *in vitro* are known as serological reactions

■ **Mention the characteristics of Ag–Ab reactions.**

- The Ag–Ab reaction is highly specific. This specificity, however, is not absolute and cross-reactions may occur with related or similar Ags
- Entire molecules react and not fragment. Though only antigenic determinant is involved in actual binding, whole molecules or particles are agglutinated
- The combination occurs at the surface; hence surface antigens participate during combination and are immunologically relevant
- The combination is firm but reversible. It is affected by affinity and avidity. **Affinity** is the intensity of attraction between antigen and antibody and **avidity** is the binding strength of the individual Ab with its specific antigenic determinant
- Ags and Abs can combine in varying proportions due to their valencies. Abs are generally bivalent and Ags may be multivalent
- Both antigen and antibody participate in the formation of the agglutinates or precipitates

■ **Mention the ways in which the presence of antigen/antibody in any system is measured.**

- Various methods used for measurement of Ag and Ab are antigen and antibody reactions such as precipitation, agglutination, complement fixation test, neutralization test, radioimmuno-assay (RIA), enzyme-linked immunosorbent assay (ELISA) or other suitable Ag–Ab reactions
- Measurement is usually in terms of units or titre
- The titre is defined as the highest dilution of the serum, which gives an observable reaction (positive reaction) with the Ag in a particular test
- Ags can also be titrated against sera (Ab)

■ Mention the important parameters of serological tests.

The two important parameters of serological tests are sensitivity and specificity.

Sensitivity

It is the ability of the test to detect even very minute quantities of Ag or Ab. When a test is highly sensitive, false negative results will be absent or minimal.

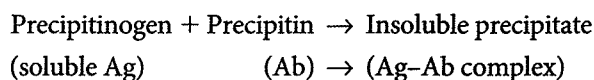
Specificity

It is the ability of the test to detect reactions between homologous Ags and Abs only. In a highly specific test, false positive reactions will be absent or minimal.

■ Describe in detail the precipitation and flocculation reactions.

Precipitation

- It is a reaction between soluble Ag (precipitinogen—Ag that has no particulate size) with its Ab (precipitin) in the presence of electrolytes at a suitable temperature (37°C) and pH (7.4) resulting in Ag–Ab complex formation as an insoluble precipitate



Flocculation

- It is a precipitation reaction in which the precipitates of Ag–Ab complex remain suspended as floccules instead of sedimentation
- Precipitation reaction can take place in a liquid medium or in semisolid medium (gels) such as agar gels, agarose or polyacrylamide gels

Zone Phenomenon

Precipitation occurs most abundantly and rapidly when Ag and Ab are present in optimum proportion. When increasing quantities of Ag are added to the fixed amount of antiserum, precipitation occurs most rapidly and abundantly in one of the middle tubes in which the Ag and Ab are present in optimum proportions. In the first few tubes in which the Ab is in excess and in the last few tubes in which the Ag is in excess, the precipitation will be minimal or absent (Fig. 19.1). If the amount of precipitate in different tubes is plotted, the resulting curve will have three different phases, viz.

1. A zone of Ab excess (prozone)—in which some uncombined Ab is present
2. A zone of equivalence—in which Ag and Ab are completely precipitated
3. A zone of Ag excess (postzone)—in which all Abs have combined with Ag but some uncombined Ag is present

This is known as zone phenomenon. Zoning occurs in agglutination and some other reactions also. The prozone is very important, as sera rich in Ab may give a false negative precipitation or agglutination reactions.

Mechanism of Precipitation

Marrack (1934) proposed a lattice hypothesis. According to this, an Ab molecule, which is divalent, forms a bridge between two Ag molecules. Ag being multivalent can combine with a number of Ab molecules. This combination results in the formation of multimolecular lattice, which makes the reaction visible. It is known as **lattice hypothesis**. This hypothesis requires that

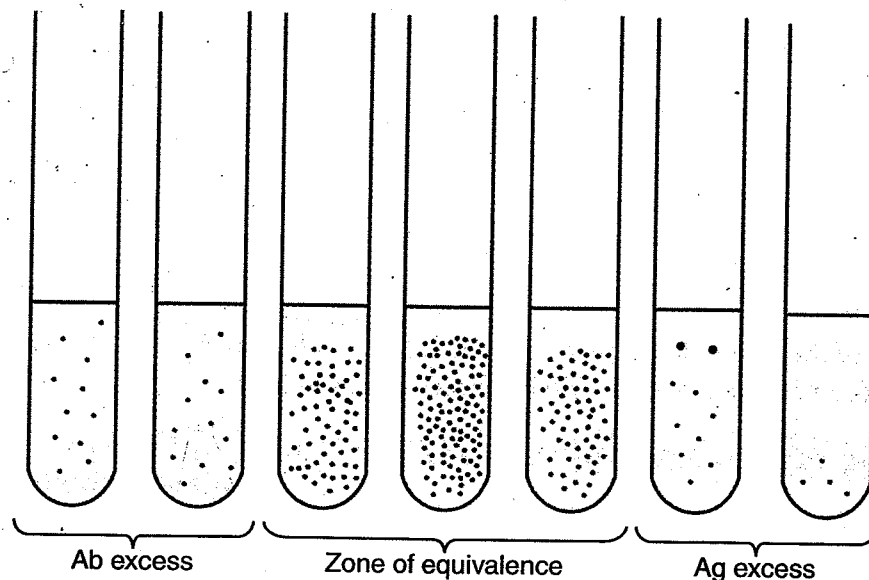


Fig. 19.1 Zone phenomenon.

- The Ab should be divalent (valency – 2 at least)
- Ag and Ab should be in optimum proportion

Precipitation occurs when Ag and Ab react in equivalent proportions (zone of equivalence). The lattice formation does not occur in the zones of Ag or Ab excess (Fig. 19.2).

Applications

- Precipitation and flocculation reactions are used for
 - Detection of Ag—more sensitive for detection of Ag and can detect as little as 1 μg of protein antigen
 - Detection of Ab—less sensitive for detection of Abs
 - Medico legal identification of human blood or seminal fluid
 - Standardization of toxins and antitoxins
- The test can be performed either as a qualitative test or as a quantitative test
- The qualitative precipitation test is widely used for detection of antigens and is particularly valuable in
 - Identification of bacteria
 - Identification of microbial components in infective tissues, e.g. *Bacillus anthracis*
 - Demonstration of antibody, e.g. VDRL (Venereal Disease Research Laboratory) test for syphilis

■ Describe the techniques of precipitation and flocculation reaction.

The following are the types of precipitation and flocculation tests:

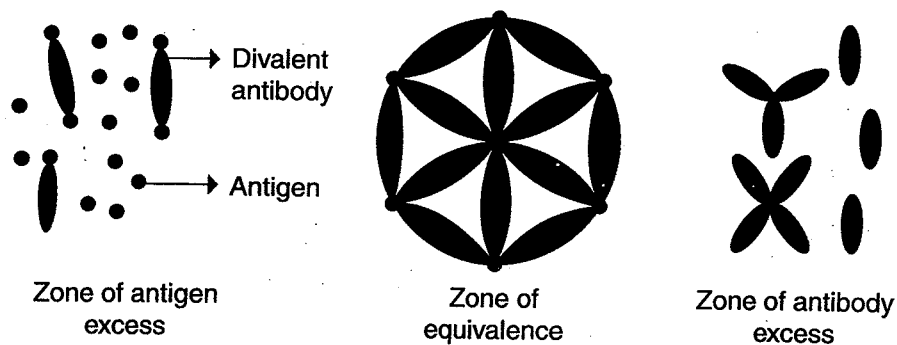


Fig. 19.2 Mechanism of precipitation.

1. Ring Test

When an antigen solution (an extract of the organism) is layered over antiserum, a white ring of precipitate forms at the junction of two fluids (Fig. 19.3).

Uses

This method is used for detection of Ag, e.g.

- Ascoli's thermoprecipitation test for anthrax
- C-reactive protein test
- Grouping of streptococci by Lancefield technique

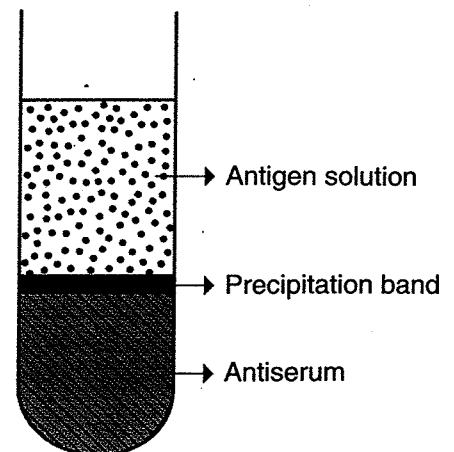


Fig. 19.3 Ring test

2. Slide Flocculation Test

When a drop of antigen solution and patient's serum are placed on a slide and mixed well by shaking, the reaction appears in the form of floccules, e.g. VDRL test for diagnosis of syphilis.

3. Tube Flocculation Test

An antigen and serum are placed in a tube and mixed by shaking—the result is formation of floccules, e.g. Kahn test for diagnosis of syphilis.

4. Immunodiffusion Test

It is precipitation in gel, which is more sensitive and specific than precipitation in a liquid medium. The advantages are:

- The gel has got certain porosity through which antigens and antibodies migrate to form precipitation band where they meet in optimum proportion. The band is stable and can be stained for better viewing and preservation
- Each antigen–antibody reaction results in formation of a distinct band of precipitation, hence the different antigens in the reacting mixture can be observed and detected
- Immunodiffusion technique also indicates identity, cross-reaction and nonidentity between different antigens

Types of Immunodiffusion

a. Single Diffusion in One Dimension (Oudin Procedure)

- In this test, the antibody is incorporated in agar gel and the antigen solution is layered over it
- The antigen diffuses downward, reacts with antibody and forms precipitation band. The number of bands indicates the number of Ags in a mixture (Fig. 19.4)

b. Double Diffusion in One Dimension (Oakley–Fulthorpe Procedure)

- In this test, both Ag and Ab move towards each other by diffusion
- The Ab incorporated in agar gel is placed at the bottom of tube
- Above this, a column of plain agar is added
- On the top of column of plain agar Ag solution is layered

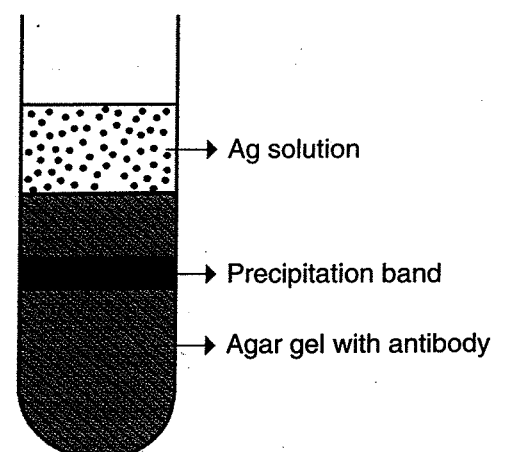


Fig. 19.4 Single diffusion in one dimension.

- Ag and Ab move towards each other through the column of plain agar and form a band of precipitate (Fig. 19.5)

Use

To determine the number of antigens in a mixture.

c. Single Diffusion in Two Dimensions (Radial Immunodiffusion)

- In this test, the Ab is incorporated in agar gel and poured on a glass slide or Petri dish and is allowed to cool and set
- Wells are cut in layer of agar
- Few wells are charged with known concentration of antigens and remaining wells with unknown Ags
- The Ag diffuses radially and reacts with Ab in gel forming ring-shaped bands of precipitation
- The diameter of the ring (halos) is directly proportional to the Ag concentration and gives an estimate of the concentration of the antigen (Fig. 19.6)

Uses

- For the quantitation of soluble Ags in body fluids
- For the quantitation of immunoglobulins, proteins and complement components in serum and other body fluids

d. Double Diffusion in Two Dimensions (Ouchterlony Procedure)

- It is also known as agar gel diffusion in which wells are punched in agar gel
- The antiserum is placed in the central well and different Ags are placed in the surrounding wells
- The slide is then placed in a moist chamber to prevent drying and to allow diffusion for 24 hours or more
- The result is formation of precipitation bands where they meet in optimum proportion
- If two adjacent Ags are identical, the lines of precipitate formed will fuse. If they are not identical, the lines will cross each other. Cross-reaction or partial identity is indicated by spur formation (Fig. 19.7)

Uses

- To detect an Ag
- To compare two Ag, Ab systems
- In the diagnosis of bacterial, viral, fungal and parasitic diseases, e.g. Elek test for toxigenicity testing in diphtheria

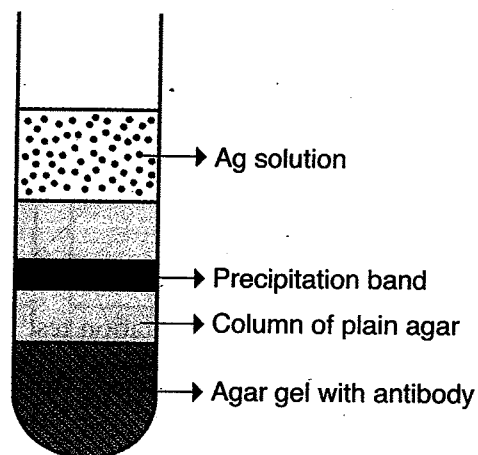


Fig. 19.5 Double diffusion in one dimension.

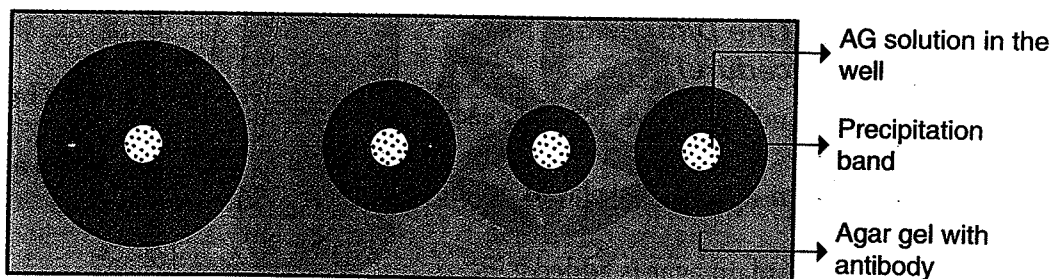


Fig. 19.6 Single diffusion in two dimensions.

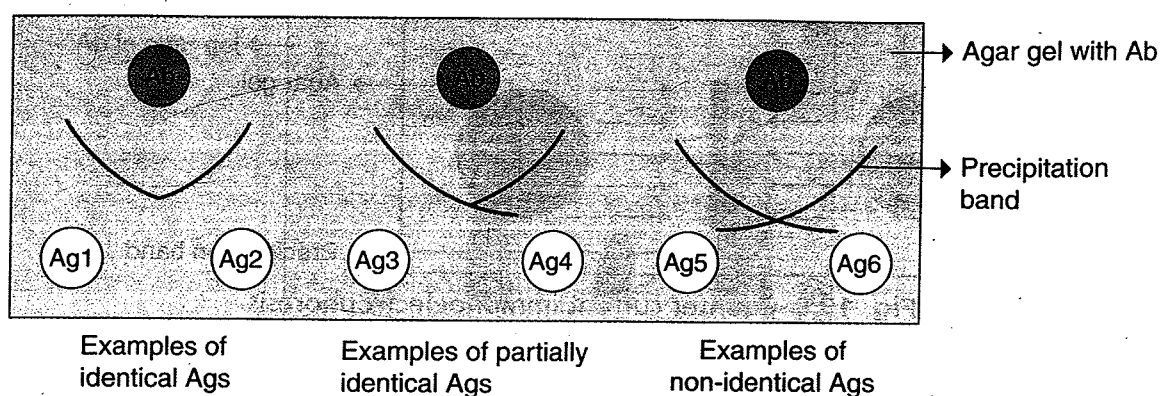


Fig. 19.7 Double diffusion in two dimensions.

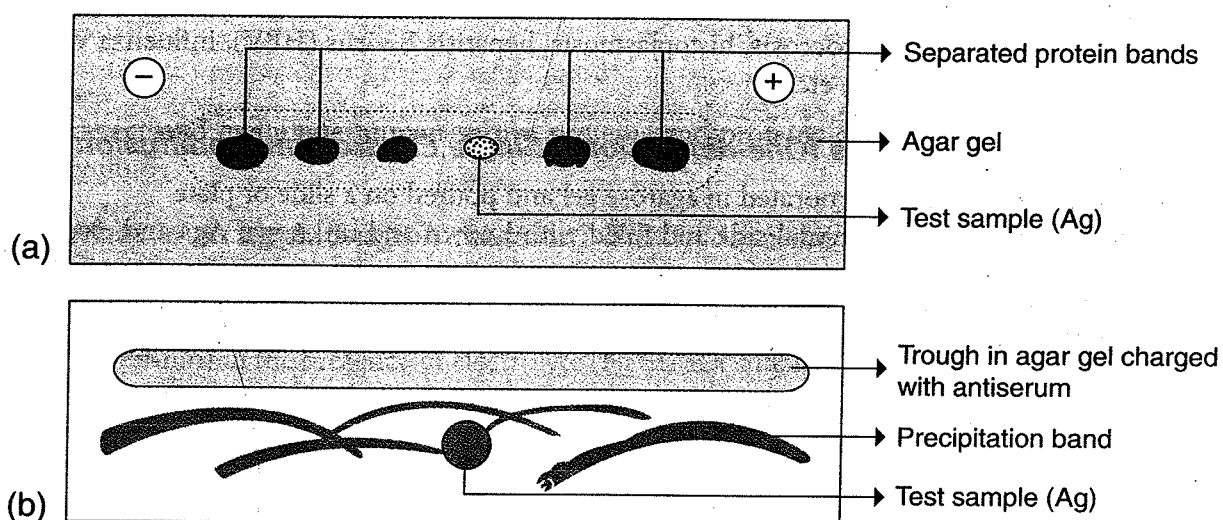


Fig. 19.8 Immunoelectrophoresis: (a) First step, (b) Second step.

e. Immunoelectrophoresis (IEP)

- It is a combination of electrophoresis and agar gel diffusion
- The test is done in two steps (Fig. 19.8):
 - In first step, the Ag is subjected to electrophoretic separation in agar gel
 - In second step, a trough is cut parallel to and slightly away from the path of electrophoretic separation and is filled with antiserum and allowed to diffuse
- The Ag and Ab diffuse towards each other and form precipitation bands

Uses

- i. Analysis of normal human serum
- ii. To detect abnormalities in human sera in diseases such as myeloma, heavy chain disease, cancer, etc. and in various body fluids

f. Counter Current Immunoelectrophoresis (CIEP) [Counter Immunoelectrophoresis (CIE)]

In this test, Ab is placed in one well and Ag to be tested in another well and are moved through gel with the help of electric current. The result is formation of line of precipitation when Ag and Ab meet in optimum proportion (Fig. 19.9).

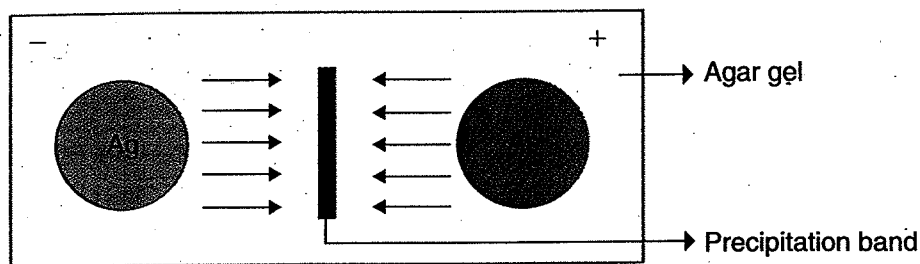


Fig. 19.9 Counter current immunoelectrophoresis.

Uses

- i. Used for detection of various Ags in various body fluids
- ii. In the diagnosis of infectious diseases such as pneumococcal, meningococcal, *C. diphtheriae*, candidiasis, cryptococcosis, histoplasmosis, hepatitis B virus (HBV), influenza virus, cytomegalovirus (CMV), etc.

g. Rocket Electrophoresis (One-dimensional Single Electroimmunodiffusion)

- In this test, the Ab is incorporated in agarose gel and poured on a slide or plate
- Wells are punched on cathodal side and filled with known and unknown Ags and electrophoresis is carried out
- The result is formation of rocket shaped bands. Based on the height of rocket, the concentration of Ag is quantitated (Fig. 19.10)

Uses

Quantitation of proteins and other Ags in various clinical specimens.

h. Two Dimensional Immunoelectrophoresis (Laurell's procedure)

This test is carried out in two steps:

- In the first step (first electrophoresis)—Ag is separated by gel electrophoresis in the different parts of gel
- In the second step—second electrophoresis is carried out at right angle to the first into Ab-containing agar gel

This causes migration of Ags into stationary Abs and results into formation of cones of precipitate. Each cone indicates one antigen (Fig. 19.11).

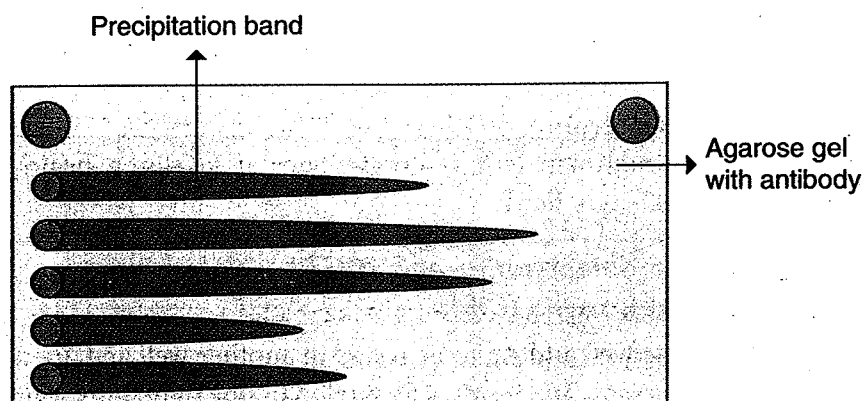


Fig. 19.10 Rocket electrophoresis.

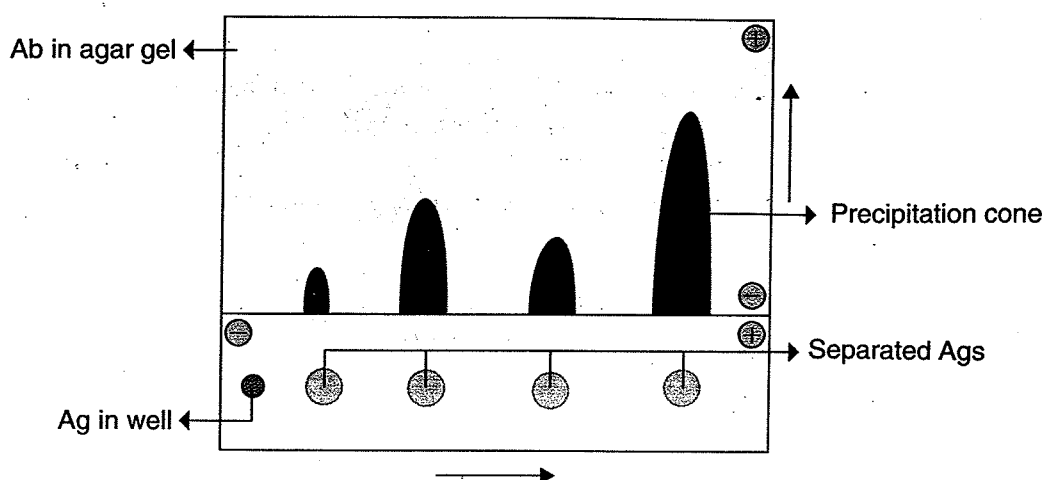


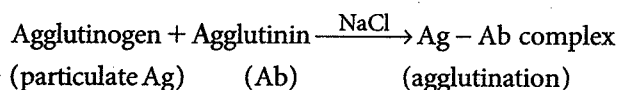
Fig. 19.11 Two-dimensional immunoelectrophoresis.

Uses

To detect and quantitate human serum proteins and proteins abnormally present in various body fluids.

■ What is an agglutination reaction? Briefly describe the procedures and uses of the various types of agglutination tests.

- It is a reaction between particulate Ag (agglutinin) such as bacteria, RBC with its Ab (agglutinin) in the presence of electrolyte (NaCl) at a suitable temperature and pH, resulting in clumping or agglutination of particulate Ag. It is more sensitive for detection of Abs



Mechanism of Agglutination

Same as precipitation reaction (lattice hypothesis).

Types of Agglutination Tests

Slide Agglutination Test

- In this test, agglutination results when to a smooth and uniform suspension of particulate Ag in a drop of saline on a glass slide or a tile, a drop of appropriate antiserum is added and mixed with a wire loop
- The clumping together of the particles and clearing of drop in a few seconds indicates positive result. Clumping is visible to the naked eye but sometimes requires confirmation under the microscope
- It is necessary to have negative control on the same slide; for this, Ag suspension is taken in a drop of saline without antiserum. If no clumping occurs, it rules out that antigen is not autoagglutinable

Uses

1. Identification of bacterial isolates from clinical specimens
2. Blood grouping and cross-matching
 - The test is rapid and requires smaller quantities of reagents but less quantitative than tube agglutination

Tube Agglutination Test

- This is carried out as a quantitative test to determine the Ab titre of serum
- In this, a fixed volume of particulate Ag suspension is added to the equal volume of serially diluted serum in test tubes and incubated
- The highest dilution of serum that gives positive agglutination reaction is recorded as Ab titre

Uses

The test is used for the diagnosis of

1. Typhoid fever (Widal test)
2. Brucellosis (Brucella agglutination test)
3. Typhus fever (Weil-Felix test)
 - The test is more quantitative than slide agglutination test but requires larger quantities of reagents
 - The problem of prozone phenomenon and presence of blocking Abs may interfere with results, hence different dilutions are tested to avoid false negative results
 - Incomplete or blocking Abs can be detected by **Coombs' antiglobulin test**

Coombs' Antiglobulin Test

Devised by Coombs et al. (1945) to detect Abs which fail to agglutinate corresponding Ag and also inhibit agglutination. These are known as **incomplete or blocking Abs**. For example, some anti-Rh-D Abs fail to agglutinate Rh-D positive red cells. It is of two types:

1. Direct Coombs' Test

- It is used to detect monovalent maternal Ab already present on the surface of RBCs
- In this test, the sensitization of RBCs with incomplete Abs takes place *in vivo*
- When such RBCs are treated with Coombs serum (rabbit antiserum) against human gamma-globulin, agglutination occurs (Fig. 19.12)

Uses

In the haemolytic diseases of newborn due to Rh incompatibility.

2. Indirect Coombs' Test

- Indirect Coombs' test is used for detection of Abs in the patient's serum
- In this test, sensitization of RBC with Ab is performed *in vitro* by incubating patient's serum with Rh-positive RBCs (group 'O' or same group RBCs) and then Coombs' serum is added
- This results into agglutination (Fig. 19.13)

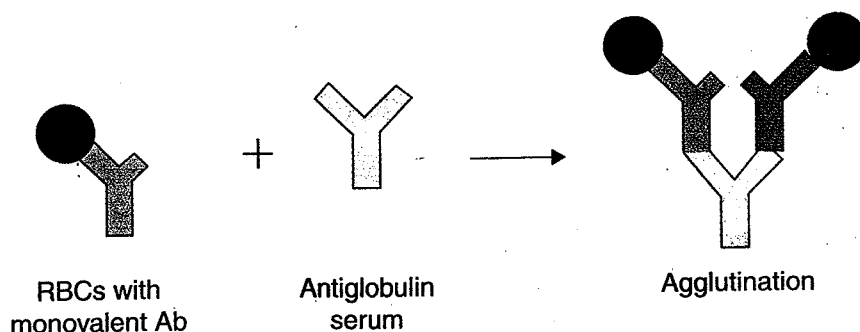


Fig. 19.12 Direct Coombs' test.

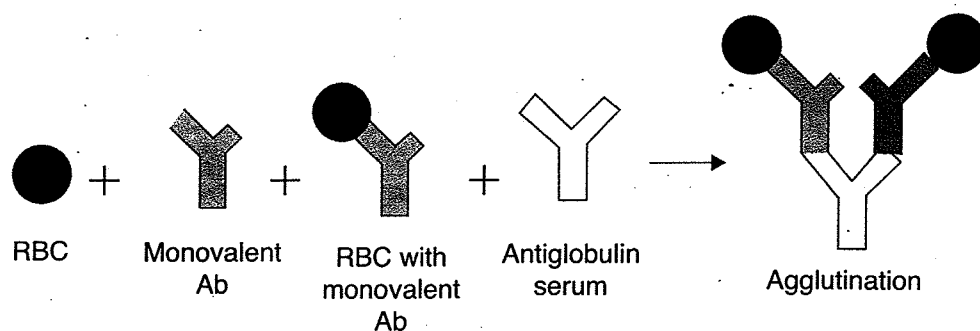


Fig. 19.13 Indirect Coombs' test.

Uses

1. The test is used for detection of anti-Rh Ab (free) in the patient's serum
2. Also to demonstrate any type of incomplete or nonagglutinating Abs, e.g. nonagglutinating Abs in brucellosis

Passive Agglutination Tests

A precipitation reaction can be converted into agglutination by coating the soluble Ag onto the surface of a carrier molecule such as latex, bentonite or RBCs. Such tests are called passive agglutination tests. When instead of Ag, Ab is absorbed on the carrier molecules for detection of Ags, the test is known as **reverse passive agglutination**. Passive agglutination tests include:

1. Latex Agglutination Test

- In this test, Ag molecules are nonspecifically adsorbed to the surface of latex particles, which have uniform diameter of 0.8–1 μ
- Addition of specific antibody transforms the latex (milk) from a milky white liquid to a coarse suspension of visible granules
- The test is rapid, convenient and specific and can be used for detection of:
 - Rheumatoid arthritis (RA) factor test
 - Pregnancy (HCG) test
 - C-reactive protein (CRP) test
 - Antistreptolysin O (ASO) test
- The test can also be used for diagnosis of cryptococcal meningitis, amoebiasis, meningococcal infections, pneumococcal infections, *Haemophilus influenzae* infections, hepatitis B virus infection and others.

2. Haemagglutination Test

In this test, the Ag is adsorbed or attached to the surface of RBC. Here, RBC acts as an inert carrier of Ag. These Ags are agglutinated by Abs, e.g. *Treponema pallidum* haemagglutination test used for the serodiagnosis of syphilis.

Co-agglutination Test

- This test uses the ability of protein A of *Staphylococcus aureus* to react nonspecifically with Fc portion of any IgG, leaving Fab portion free to react with its homologous Ag
- The IgG on protein A of *Staph. aureus* reacts with specific Ag that causes clumping of the staphylococci
- This clumping or agglutination of *Staph. aureus* is called co-agglutination (Fig. 19.14)

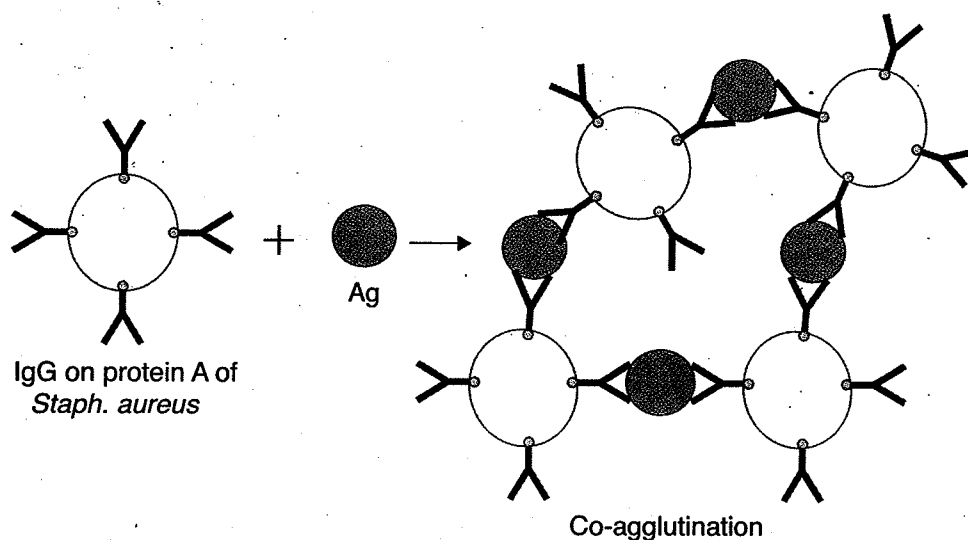


Fig. 19.14 Co-agglutination test.

Uses

The test is used for detection of bacterial Ags in blood, urine and cerebrospinal fluid (CSF) in pneumococcal, gonococcal, meningococcal, streptococcal, *Salmonella* and *Haemophilus* infections.

■ Describe in detail the complement fixation test (CFT).

- The ability of Ag-Ab complexes to fix complement is used in CFT. It is a versatile and sensitive test capable of detecting as little as 0.04 μ of Ab nitrogen and 0.1 μ of Ag
- Described by Bordet and Gengou (1901)

Requirements

- Complement—It is obtained from guinea pig serum
- Patient's serum—5 ml of blood is collected. Serum is separated and heated at 56°C for 30 minutes to destroy the complement present
- Suitable, soluble or particulate Ag obtained from commercial source or prepared in laboratory
- Sheep red blood cells
- Amboceptor (rabbit or horse antish sheep RBC serum)—rabbit or horse is immunized with sheep RBCs, so that it forms Abs against sheep RBCs. These Abs are called amboceptors

Procedure

It is a two-step test:

Step 1

Inactivated patient's serum + Ag + Fixed amount of complement
Incubated at 37°C for 60 minutes

Step 2

Sheep RBCs sensitized with anti-sheep RBC Abs (amboceptor) are added and incubated at 37°C for 30 minutes (Fig. 19.15).

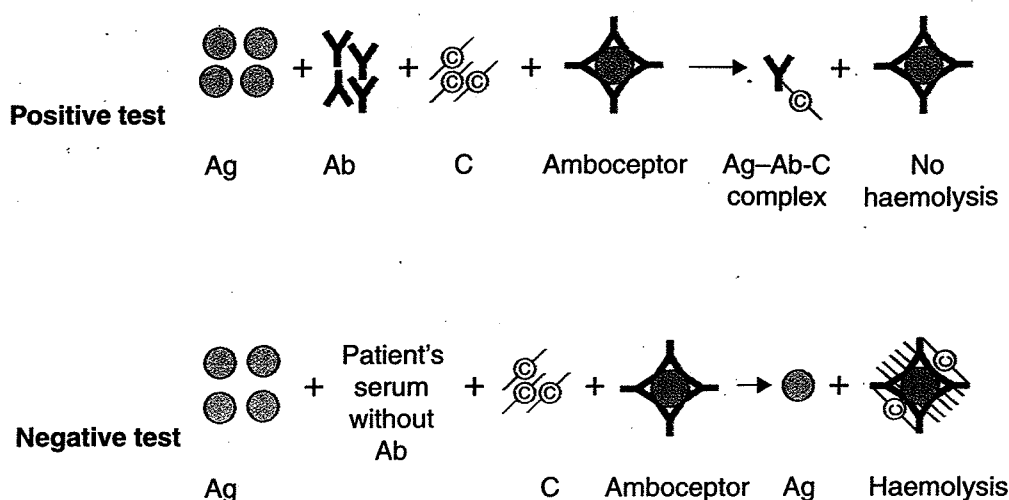


Fig. 19.15 Complement fixation test.

Results

- The test is considered positive if there is no haemolysis of sheep RBCs
- The test is considered negative, if there is haemolysis of sheep RBCs
- Example: Wasserman test for diagnosis of syphilis
- In a positive test, the patient's serum contains Abs, which react with Ags and fix complement. Thus, complement is utilized in first step and there is no free complement present for lysis of sheep RBCs (SRBCs)
- In a negative test, the serum does not contain Abs hence there will be no Ag–Ab reaction and complement will not be utilized in first step. It will be left intact, which reacts with SRBCs and causes haemolysis

Indirect Complement Fixation Test (Indirect CFT)

- Certain avian (duck, turkey, parrot) and mammalian (horse, cat) sera do not fix guinea pig complement. In such cases, indirect CFT is used. The test is set up in duplicate
- In first step, Ag + Test sera + Complement are added
- To one set a standard antiserum known to fix complement is added and finally sheep RBCs with Abs are added

Result

- In this test, if Abs are present in test serum, they react with Ag, hence standard antiserum will not react with Ag and complement will not be fixed. Thus, the complement causes haemolysis, which is considered as positive test
 - If Abs are not present in test serum, the standard antiserum will react with Ag and complement will be fixed so that there will be no haemolysis that indicates negative test
- Indirect complement fixation test can be summarized as:

Ag + Test serum + C + Standard antiserum + SRBCs + Abs—
 Haemolysis—Positive test
 No Haemolysis—Negative test

■ Write an account on the other complement dependent serological tests.

The other complement dependent serological tests are as follows:

1. Immobilization Test

- In this test, a live motile suspension of *Treponema pallidum* is mixed with patient's serum and complement is added and is observed for motility
- The test is considered positive, if the motility is inhibited and the organisms become nonmotile, e.g. *T. pallidum* immobilization test.

2. Immune Adherence Test

Bacteria such as *Vibrio cholerae* and *T. pallidum* when react with the specific Ab, adhere to the surface of particulate materials such as RBCs, platelets or macrophages in the presence of complement. This is known as immune adherence.

3. Cytocidal or Cytolytic Test

When live bacteria such as *V. cholerae* are mixed with specific Ab in the presence of complement, the bacterium is killed and lysed (vibriocidal Ab test).

■ Enumerate the applications of CFT.

CFT is used in the diagnosis of

- Bacterial infections such as *T. pallidum*, *V. cholerae*, *Brucella*, *Bordetella*, *Chlamydiae*, *Rickettsiae*, *Mycoplasma*, etc.
- Viral diseases such as herpes, hepatitis, mumps, polio, etc.
- Parasitic diseases such as leishmaniasis, amoebiasis, giardiasis, malaria, hydatid disease, etc.
- Fungal diseases such as histoplasmosis, blastomycosis, etc.

■ Describe neutralization test.

Homologous Abs are able to neutralize the biological effects of viruses, toxins and enzymes. Such Abs are known as neutralizing Abs and the test is known as neutralization test. Neutralization test is of two types:

1. Virus Neutralization Test

Various methods are available to demonstrate virus neutralization. These include animals, tissue culture and chick embryos. The inoculation of virus in these systems results in growth and multiplication. Inhibition of growth and multiplication by injecting or incorporating specific neutralizing Abs indicates positive neutralization test (Fig. 19.16).

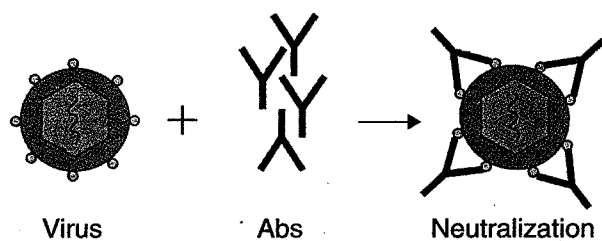


Fig. 19.16 Virus neutralization test.

The neutralization of bacteriophages can be demonstrated on a lawn culture of susceptible bacteria. When a phage is applied on the lawn culture, it causes lysis of bacteria and forms plaques, but when specific antiphage antiserum is added to culture, formation of plaque is inhibited because of neutralization.

2. Toxin Neutralization Test

When antitoxin containing homologous Abs combines with its toxin, it neutralizes biological effects of toxin making it harmless. Toxin neutralization can be studied *in vivo* and *in vitro* (Fig. 19.17).

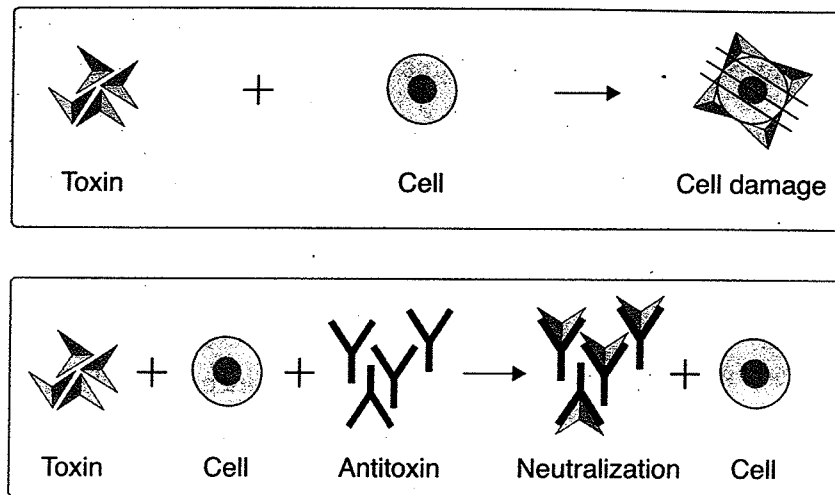


Fig. 19.17 Toxin neutralization test.

(A) Toxin Neutralization Test *in vivo*

Example: Schick test to determine immunity or susceptibility to diphtheria. In this test, when diphtheria toxin is injected intradermally in a human, no reaction occurs at the site of injection, if the individual possesses circulating antitoxin in his blood.

Other examples of *in vivo* neutralization tests are:

- Toxigenicity testing of *C. diphtheriae* in experimental animals
- *Cl. welchii* toxin neutralization test in guinea pig or mice

(B) Toxin Neutralization Test *in vitro*

- Antistreptolysin O test:** In this test, antitoxin present in patient's serum neutralizes streptolysin O and inhibits its haemolytic activity
- Nagler's reaction in *Cl. welchii*:** Toxin is neutralized by antitoxin when bacteria are grown in egg yolk medium containing antitoxin (Fig. 35.2)

■ Describe immunofluorescence. Highlight the features of immunofluorescence tests and mention two uses of each test.

Fluorescence is the property of absorbing light rays of one particular wavelength and emitting rays of a different wavelength. Fluorescent dyes can absorb ultraviolet light and convert it into visible light. Fluorescent dyes such as fluorescein isothiocyanate and lissamine rhodamine can be conjugated to Abs and used to locate and identify Ags in tissues (Coons and Kaplan, 1950). The method can also be used for detection of antibodies directed against Ags already known to be present in a given specimen. This test is more sensitive than precipitation or CFT. The test can be performed as:

1. Direct Immunofluorescence Test

- In this test, the tissue section or smear of clinical specimen is fixed on a glass slide and specific Ab tagged with fluorescent dye is applied directly to slide
- It is allowed to react for 30–60 minutes at 37°C and observed for fluorescence under UV microscope (Fig. 19.18)

Uses

1. Commonly used for detection of bacteria, viruses, fungi and parasites in tissues, blood or other specimens
2. Used for the identification of tumour Ags, enzymes, hormones, organ and tissue Ags, blood cells, etc.

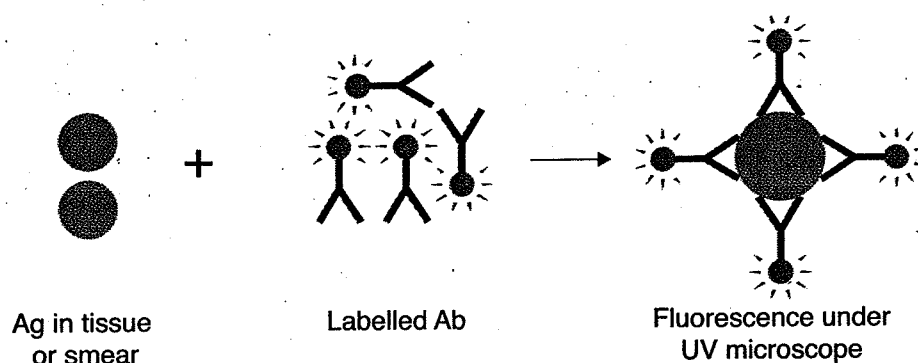


Fig. 19.18 Direct immunofluorescence test.

2. Indirect Immunofluorescence Test

- It is used for detection of Abs in sera or other body fluids. It is a double layer technique
- In this test, Ag is known. It is treated with test serum, if Abs are present in serum a complex is formed. This complex is visualized by a fluorescent dye tagged with anti-immunoglobulin serum (Fig. 19.19)

Uses

1. Used to detect Abs in *T. pallidum*, *Brucella*, pneumococci, *Cryptococcus*, cytomegalovirus, rubella virus, malaria, *Echinococcus* and other infections
2. In the diagnosis of cancer, autoimmune diseases, etc.

3. Sandwich Test

This is a double layer technique used to visualize specific Ab. In this test, Ab is first allowed to react with unlabelled Ag, which is then treated with fluorescent labelled Ab. Thus, Ag is sandwiched between labelled and unlabelled Abs. Used for detection of Abs.

■ Explain the technique of radioimmunoassay (RIA). Briefly mention its procedure and uses.

RIA uses radioactive isotopes to detect Ags or Abs. The test is described by Berson and Yalow (1960). It is used as sensitive and specific method for quantitation of hormone, drugs, viral Ags, etc. It permits the measurement up to picogram (10^{-12} gram) quantities.

It is basically a competitive binding assay in which fixed amounts of Ab and radiolabelled Ag react in the presence of known (standard) or unknown (test) amounts of the Ag. The amount by which the binding of labelled Ag to its Ab is competitively inhibited by increasing amounts

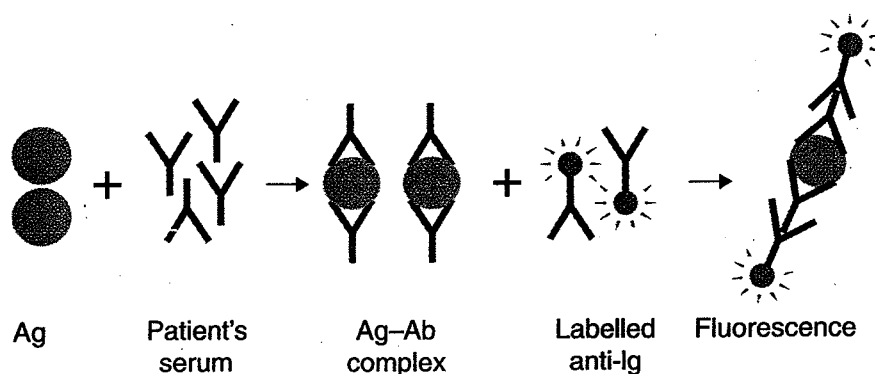


Fig. 19.19 Indirect immunofluorescence test.

of standard Ag preparations are recorded. A dose response curve or standard curve is plotted and the amount of Ag present in unknown sample can be calculated from the standard curve by comparing with the binding of labelled Ag.

Procedure

- The Ag is radiolabelled and it is reacted with enough Ab to bind about 70% of it
- Various known amounts of unlabelled Ag are added to allow competition for Ab
- The labelled Ag bound to Ab is separated from unbound labelled Ag by electrophoresis, chromatography, gel diffusion or double Ab method
- From amounts of labelled Ag bound at various concentrations of unlabelled Ag, a standard curve is constructed which allows quantitation of unknown Ag in the test sample

Uses

RIA can be used for quantitation of hormones, drugs, tumour markers, IgE, viral Ags, autoimmune markers, etc.

■ Write a note on enzyme-linked immunosorbent assay (ELISA).

It is a type of binding assay that depends on Ag–Ab reaction as base and enzyme reaction as marker. This is a simple, versatile and highly sensitive test and needs only microlitre quantities of test reagents. It is widely used for detection of a variety of Abs and Ags. The test may be performed in polystyrene tubes (macro ELISA) or in polyvinyl microtitre plates (micro ELISA).

1. Sandwich ELISA

It is used for assay of an Ag. In this test,

- The wells of microtitre plate are coated with specific Ab against Ag to be tested
- The sample to be tested is added to the well and incubated. If Ag is present in test sample, it reacts with Ab. To detect this Ag–Ab reaction, Ab labelled with an enzyme is added and incubated. This Ab labelled with enzyme binds to an Ag
- After washing when suitable substrate is added, the enzyme acts on substrate forming the product
- The enzyme activity is measured by spectrophotometric or fluorometric or electrode method (Fig. 19.20)

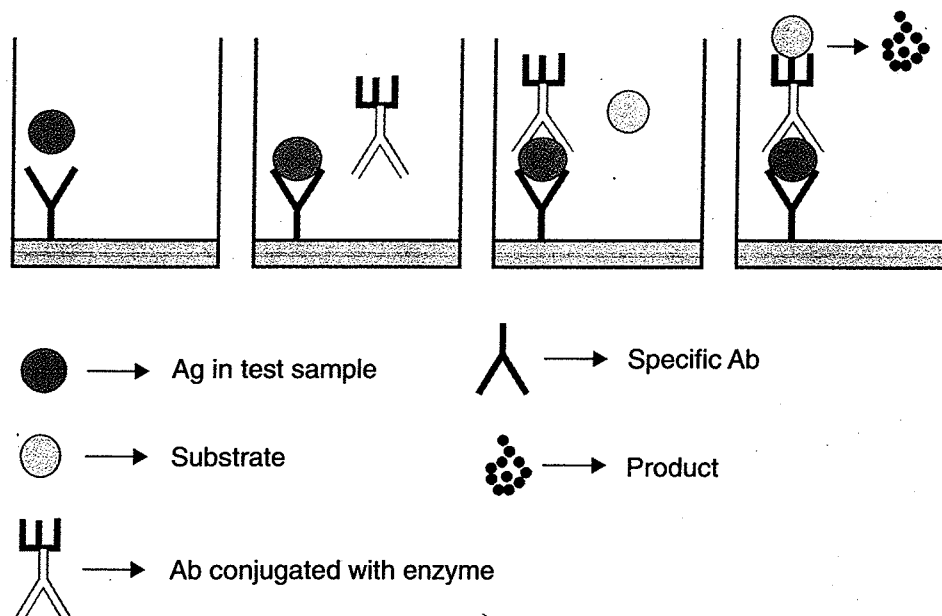


Fig. 19.20 Sandwich ELISA.

If the test sample contains specific Ag, it is fixed to the Ab coating the wells. When the enzyme-labelled Ab is added subsequently, it gets fixed on Ag and the presence of enzyme activity is indicated by the development of yellow colour, which indicates positive test. If the sample is negative for Ag there will be no change in colour.

Uses

1. Detection of Ags such as rotavirus Ag, hepatitis B virus Ag, etc.
2. Detection of hormones and toxins

2. Indirect ELISA

It is used for detection of Abs. In this test,

- Ag is coated in well instead of Ab and treated with patient's serum
- A goat antihuman immunoglobulin Ab conjugated with an enzyme is added
- A suitable substrate is added, the enzyme acts on substrate forming the product
- The enzyme activity is measured by spectrophotometric or fluorometric or electrode method (Fig. 19.21)

Uses

1. Detection of Abs in various infections such as *Salmonella*, *Haemophilus*, *V. cholerae*, *Brucella*, *Treponema*, Rubella, HIV, Hepatitis-B virus, Herpes simplex virus, Cytomegalovirus
2. Detection of anti-DNA Abs in systemic lupus erythematosus

■ Explain the technique of immunoblotting.

Immunoelectroblot or electroimmunoblot is a highly sensitive and a highly specific test to detect individual proteins (Ags) in complex mixtures. It is a combination of three different procedures:

- **Separation:** The mixture of proteins (Ags) is electrophoretically separated by polyacrylamide gel electrophoresis
- **Blotting:** The separated proteins are then blotted or transferred electrophoretically from polyacrylamide gel to a sheet of nitrocellulose paper

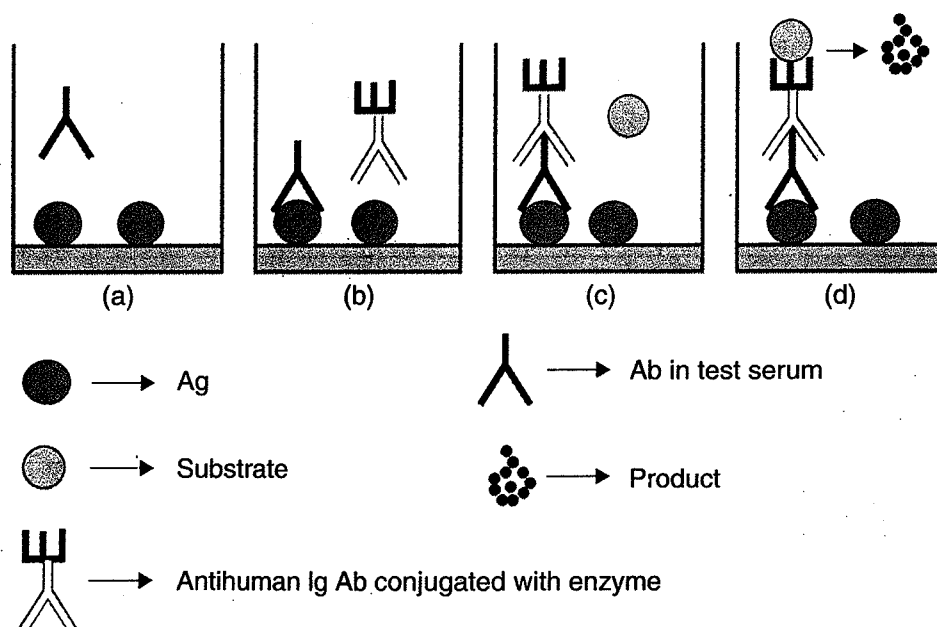


Fig. 19.21 Indirect ELISA.

- **Enzyme immunoassay** (or radioimmunoassay) to detect Ab in test sera against various Ag fractions

Western blot

It is the confirmatory test used for serodiagnosis of HIV infections. In this test,

- The proteins (Ags) are separated by polyacrylamide gel electrophoresis based on their electrophoretic mobility and molecular weight
- The separated proteins are blotted onto nitrocellulose paper
- These strips are then treated with test sera and finally with enzyme conjugated antihuman globulin
- A suitable substrate is then added which produces a prominent colour band where the specific Ab has reacted with the separated Ag
- The position of the band on the strip indicates the Ag with which the Ab has reacted

■ **Explain the various immunoelectron microscopic tests.**

Some commonly used immunoelectron microscopic tests are:

1. Immunoelectron Microscopy

Ab combining with viral particles causes clumping of viruses, which can be visualized by electron microscope. This method is used for viruses such as Hepatitis-A virus and viruses causing diarrhoea.

2. Immunoferritin Test

Ferritin (electron-dense substance from horse spleen) can be conjugated with Ab and used to trace intracellular virus Ags, which can be visualized under the electron microscope.

3. Immunoenzyme Test

Some stable enzymes such as peroxidase can be conjugated with Abs and used to visualize the Ag in tissue sections under the electron microscope by microhistochemical methods.

20

Chapter

The Complement System

■ Describe the complement system.

The complement system refers to an extremely complex group of proteins present in normal human and animal serum. It is an integral part of the body's immune system that has ability to

- Lyse red blood cells (haemolytic activity)
- Destroy Gram-negative bacteria (bacteriolytic activity)
- Kill Gram-positive bacteria without lysis (bactericidal activity)
- Inactivate viruses (virus neutralization)
- Damage tumour cells

Ag-Ab complexes absorb it nonspecifically and mediate a number of immunological and biological activities. Paul Ehrlich named this lytic agent as **complement** as it complemented the action of antibody. Earlier it was known as **Alexine**.

■ Describe the nature of complement.

- Chemically, complement is a group of proteins, complex in nature, associated with some carbohydrates and phospholipids
- The complement system consists of approximately 20 serum proteins
- These include:
 - Complement components (C1 to C9)
 - Properdin system
 - Regulatory proteins—these are enzymes, control molecules and structural proteins without any enzymatic activity

■ Mention the properties of complement.

Properties of complement are:

- Complement is a **glycoprotein**—constituting approximately 5% to 10% of human serum proteins
- Though some of the individual components are heat stable, **complement as a whole is heat labile**. It undergoes spontaneous denaturation slowly at room temperature and in 30 minutes at 56°C (inactivation)
- It **does not combine with Ag or Ab alone**
- It is **destroyed by trypsin**

■ Mention the nomenclature of complement components.

- Complement components are designated as C1 to C9
- The product of reaction designated as Ag-Ab-C, e.g. Ag-Ab-C1423
- Components, which acquire enzymatic or biological activity, are indicated by a bar over the components, e.g. $\overline{C1}$ is the enzymatically-activated form of C1

- Fragments of C cleaved during the reaction are indicated by lower case letters, e.g. C3a, C3b
- Inactivated forms of C components are indicated by the suffix 'i', e.g. C3i

■ Enumerate the components of complement.

- Complement is not a single substance. It is a complex of different protein fractions. Electrophoretic separation shows nine distinct proteins, C1 to C9
- C1—heat labile, forms the main bulk of the complement. It is made up of three protein subunits, *viz.* C1q, C1r and C1s bound together by one molecule of calcium
- C2—heat labile component
- C3—heat stable component, a relatively larger protein which has the ability to combine with yeast cell wall (Zymosan)
- C4—heat stable, susceptible to ammonia and hydrazine
- C5 to C9—These are the terminal components of membrane attack pathway required for the final show of immune cytolysis

■ How is a complement activated?

- Complement is normally present in an inactive form
- It is activated sequentially in an enzyme cascade manner by Ag-Ab complexes (classical pathway) or other stimuli (alternate pathway)
- The initial steps are different in different pathways, the final steps that lead to a membrane attack which produce damage to target cell or lysis causing cell death are same in both pathways

■ Discuss the pathways by which a complement is activated.

The pathways by which a complement is activated are: the classical pathway and the alternate pathway.

The Classical Pathway (Fig. 20.1)

- This pathway involves operation of all nine components (C1 to C9) acting in sequence
- It begins with the activation of C1q (first component) initiated by binding with CH2 domain on the Fc portion of Ab forming complex with Ag
- Fc structures are different in different immunoglobulins and only IgG and IgM possess complement-binding sites
- When the C1q is activated, it acquires the ability to activate several molecules of the next component in a series. Each of these then acts on next component and so on, producing a cascade effect leading to immune cytolysis

The Cascade Effect

- The activation of complement starts when IgM or IgG combines with the surface membrane of the cell in the presence of complement
- C1q then binds to Fc portion of Ig molecule and activates C1r, which is a protease that subsequently cleaves C1s and forms an enzymatically active component C1s esterase
- The activated C1s acts on the next component—C4 splitting it into C4a and C4b. The C4a has anaphylatoxin activity while C4b, which has an active site, binds to cell membrane along with C1
- The next component C2 with the help of Mg^{2+} ions binds to the cell bound C4b and is cleaved into C2a and C2b
- C2b is released into fluid medium while C2a binds to the C4b forming C4b2a protease enzyme, which has 'C3 convertase' activity, which cleaves C3 into C3a and C3b
- C3a released into the fluid medium possesses anaphylatoxin activity and is chemotactic for polymorphonuclear leucocytes and C3b joins the cascade and binds to the surface of the

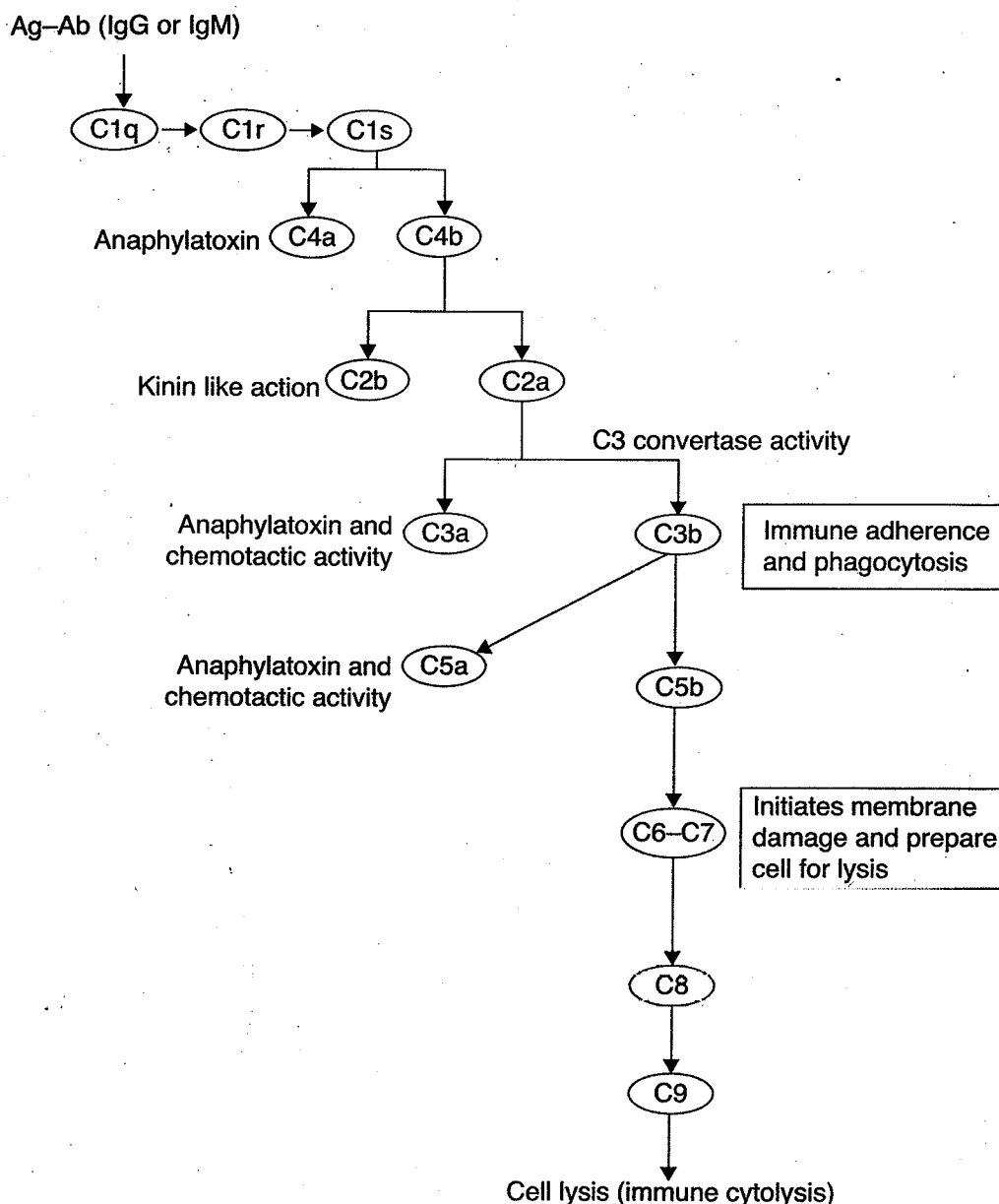


Fig. 20.1 Classical complement pathway.

target cell along with C4b2a forming a trimolecular complex C4b2a3b, which has C3 convertase activity

- C3b fragments not joining the cascade are scattered over the cell membrane, participate in immune adherence and increase susceptibility to phagocytosis (opsonization)
- C3 convertase acts on C3, splits it into C3a and C3b. C3a has anaphylatoxin and chemotactic activity and larger fragment C3b joins the cascade and binds to C4b and C2a forming a trimolecular complex C4b2a3b which binds to the cell membrane of target cell and prepares it for lysis by C5 and C6
- The addition of C5 and C6 accelerates membrane damage and lysis of the cell
- The mechanism of lysis is by punching holes approximately 100 Å in diameter on the cell membrane which disrupts the osmotic integrity of the membrane leading to release of the cell contents
- The complex C5b67 also renders unsensitized 'bystander' cells susceptible to lysis by C8 and C9. The complex also has chemotactic and leucocyte activating properties

The Alternate Pathway (Fig. 20.2)

- C3 is the major component of C and its activation is the central process in complement activation. In the classical pathway, C3b is formed by the action of C142, which has got C3 convertase activity. The alternate pathway bypasses participation of C1, C4 and C2, and activates C3 directly. This activation of C3 without prior participation of C1, C4 and C2 is known as the alternate pathway
- Pillemer (1954) described the alternate pathway. He demonstrated alternate pathway of the properdin, which combines with zymosan (a yeast cell wall polysaccharide) in the presence of Mg^{2+} ions and forms P-Z complex, which activates C3 directly without the help of C1, C4 and C2

Important Components of Alternate Complement Pathway

- **Properdin (P)**—glycoprotein normally present in serum, requires Mg^{2+} ions and other factors for activation of C3
- **Factor-B**—thermolabile normal serum protein, it is C3 proactivator (C3PA) also known as glycine rich betaglobulin (GBG)
- **Factor-D**—C3 proactivator convertase (C3 pase, GBGase), it acts on C3b and factor-B forming convertase, which breaks down the C3
- **C3b**—normally present in serum in trace amount

Activation of C3

- The factor-B binds to the trace amount of C3b that exists in normal serum and forms a magnesium dependent C3bB complex, which is cleaved by a proteolytic enzyme, factor-D into C3bBb and Ba
- C3bBb complex has C3 convertase activity but it is extremely labile and loses its activity; but by binding with properdin it forms PC3bBb complex and becomes stable
- PC3bBb acts on C3 cleaving it into C3a and Disease Research Laboratory activating C6, C7, C8, C9
- The steps are same as in classical pathway

Triggering Agents

In alternate pathway C3 can be triggered by substances such as:

- Microbial polysaccharides—zymosan, inulin, lipopolysaccharide of Gram-negative bacteria (endotoxins), etc.
- Aggregated immunoglobulins such as human IgG4 and IgA, which are unable to bind C1q and activate classical pathway
- Microorganisms such as influenza and other viruses, cryptococci and other fungi
- Snake venom factor

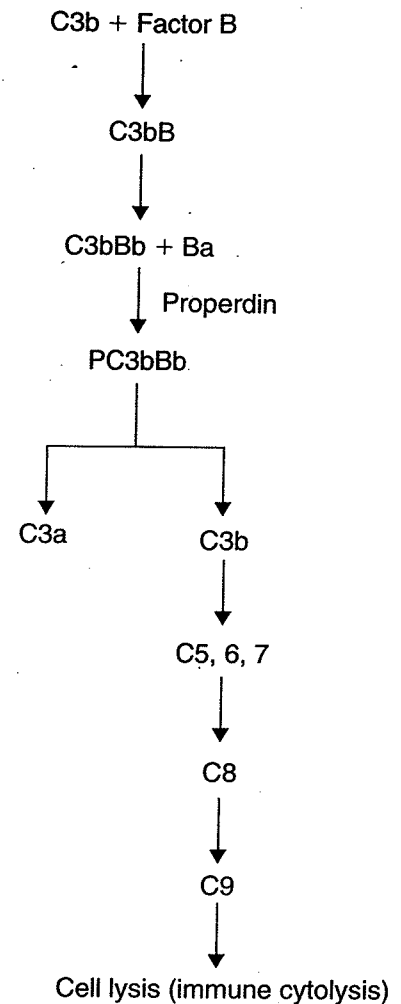


Fig. 20.2 Alternate complement pathway.

■ Discuss regulation of C activation.

Activation of the complement cascade results in a complex series of molecular events with potent biological consequences. Unchecked activation may cause:

- Exhaustion of the complement system
- Serious damage to tissues

Hence, regulation of complement activation is necessary to control the production of biologically active split products. The cascade is regulated by several inbuilt control mechanisms at different steps. These are mainly of two types:

- Inhibitors, which bind to C components and halt further function
- Inactivators, which are enzymes that destroy, complement proteins

Inhibitors

- C1 esterase inhibitor (C1sINH): A heat labile α -neuroaminoglycoprotein present in normal serum. It is a regulatory protein that inhibits C1 esterase (inhibitor of C1)
- The S protein: It is normally present in serum, binds to C567 and prevents their insertion into cell membrane modulating the cytolytic activity of the membrane attack complex

Inactivators

- Factor-I: It is a serum β -globulin that cleaves and inactivates C3b and C4b and controls C3 activation, particularly by the alternate pathway
- Factor-H: It is another β -globulin factor that acts in cooperation with factor-I and modulates C3 activation
- C4 binding protein: It controls the activity of cell bound C4b
- Anaphylatoxin inactivator: It is an α -globulin that enzymatically degrades C3a, C4a and C5a which are anaphylatoxins released during the complement cascade
- Many other regulators such as decay-accelerating factor (DAF), homologous restriction factor (HRF), membrane cofactor protein (MCP), etc. have been reported to modulate the activity of the complement

■ Mention the ways in which the complement nonidentity in immunological and biological activities.

The complement system plays an important role in various immunological and biological activities. These include:

- **Neutralization of viruses:** particularly in the early stages of infection by either pathway
- **Chemotaxis:** C5a, C3a and a trimolecular complex C567 are chemotactic for polymorphonuclear leucocytes (PML). They attract PML at the site of Ag-Ab interaction and enhance phagocytosis
- **Bactericidal activity:** The complement renders Gram-negative bacteria susceptible to lysozyme by digesting holes in lipopolysaccharide layer which protects inner lysozyme sensitive layer of peptidoglycan and thus makes Gram-negative bacteria sensitive to lysis (bacteriolysis). Gram-positive bacteria are not susceptible to the lytic action because of their cell wall composition. However, they are killed by complement without lysis
- **Immune adherence:** The binding of C3b to Ag-Ab complexes, Ab sensitized cells and viruses make them to adhere to cells possessing immune adherence receptors (PML, macrophages, some B lymphocytes, primate RBCs, etc.) and increase susceptibility to phagocytosis

- **Opsonization:** Components of C (C1, C4, C2, C3 and C5) enhance the phagocytosis of immune complexes. This is complement dependent opsonization important in limiting bacterial infections
- **Anticancer activity:** It has been shown to cause death of tumour cells
- **Anaphylatoxin activity:** C3a, C5a, C4b and C2 kinins stimulate the release of histamine from mast cells that causes contraction of smooth muscles, increases vascular permeability and vasodilatation
- **Tissue destruction:** Activation of complement also causes severe tissue destruction in autoimmune diseases such as autoimmune haemolytic anaemia, thrombocytopenia, myasthenia gravis, Good Pasteur's syndrome, etc. and in hypersensitivity reactions such as Arthus reaction and serum sickness
- **Conglutination:** Bovine serum contains β -globulin component called conglutinin (κ), which causes clumping of particles or cells with complement. This process is known as conglutination.
- **Haemolysis:** It causes lysis of RBCs by activating classical pathway
- It also participates in coagulation of blood

■ **Name the sites in the human body where components of C are synthesized.**

The components of C are synthesized at various sites in the body such as:

- C1—intestinal epithelium
- C2 and C4—macrophages
- C5 and C8—spleen
- C3, C6 and C9—liver

21

Chapter

Structure and Functions of Immune System

- Name the important organs of immune system and describe in short their role in the development of immunocompetent cells.

The immune system comprises a variety of organs which are concerned with growth and development of immunocompetent cells. These are lymphoid and reticuloendothelial systems (lymphoreticular system; Flowchart 21.1).

Lymphocytes are the major immunological effector cells. They arise from precursors or stem cells, which originate in fetal life in the yolk sac and are found subsequently in the liver and bone marrow. They are further differentiated in two different directions. Some of them migrate to thymus and get differentiated into thymus derived or T lymphocytes, which play an important role in cell-mediated immunity. Other stem cells are processed in the bone marrow or the bursa of Fabricius (in chicken) and get differentiated into B lymphocytes, which play an important role in humoral or antibody-mediated immunity.

Both T and B cells, after maturation, migrate to spleen, lymph nodes and other organs where they initiate and participate in immune response to antigens.

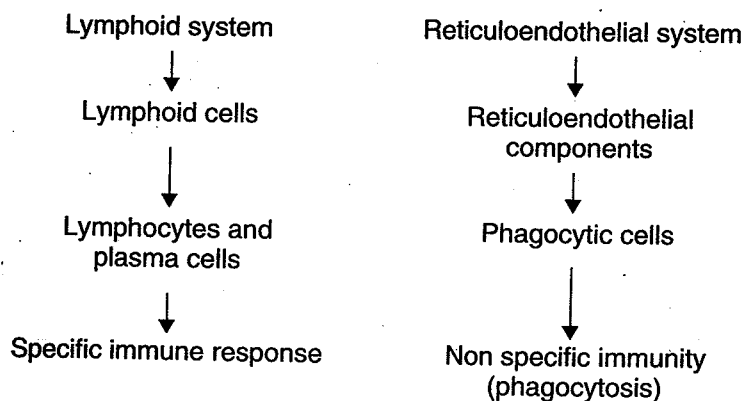
The development of principal cells of immune system is shown in Fig. 21.1.

The lymphoid system consists of

- Lymphoid organs
- Lymphoid cells (lymphocytes and plasma cells)

Types of lymphoid organs—two types based on different functions they perform:

1. The central (primary)
2. The peripheral (secondary) lymphoid organs



Flowchart 21.1 Lymphoreticular system.

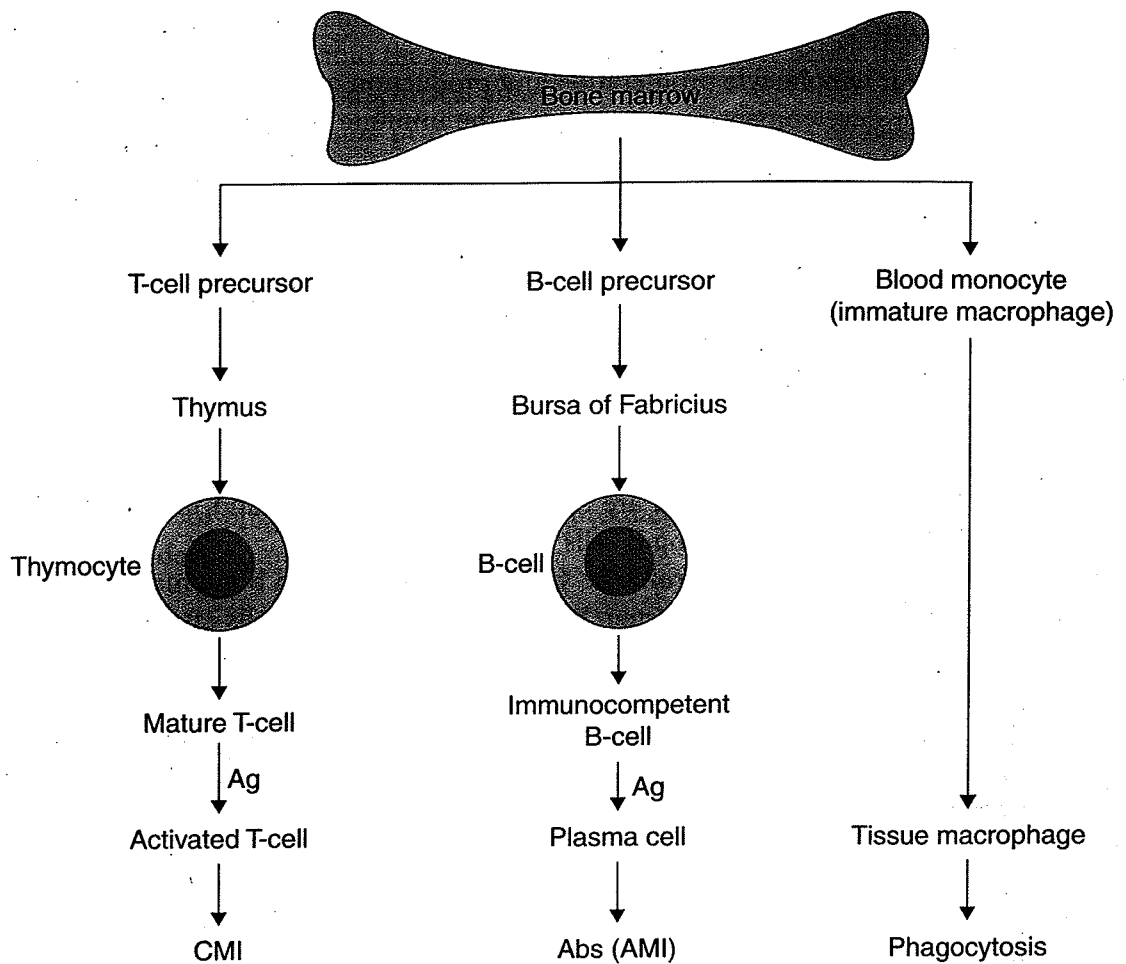


Fig. 21.1 Development of principal cells of the immune system.

■ **Describe the organs of the central (primary) lymphoid organs. Also enumerate their functions.**

These are the lymphoid organs in which proliferation and differentiation of lymphocytes takes place without antigenic stimulation (antigen independent maturation of lymphocytes). The primary lymphoid organs include:

1. Thymus

- It is a small lymphoepithelial bilobed organ in the anterior mediastinum
- Derived from the third and fourth pharyngeal pouches and differentiated from these pouches at about the sixth week of fetal life
- Acquires characteristic lymphoid appearance by the third month of gestation
- It is capsulated. The septa arising from the capsule divide the gland into lobules, which are differentiated into an outer cortex and inner medulla
- The precursors of lymphocytes from yolk sac, fetal liver and bone marrow reach the thymus and mature in the cortex, acquire surface characteristics of T lymphocytes and then migrate into medulla where they complete maturation process and exit into the blood as matured T cells capable to responding to antigenic stimuli and seeded into the secondary lymphoid organs
- Mature thymocytes in thymus are about 5–10% of the total population

Functions of Thymus

- It is a major site for lymphocyte proliferation and production of T lymphocytes
- It is the centre for development and function of the immune system; however, it does not participate in immune reaction
- In thymus, lymphocytes acquire new surface antigens (Thy antigens)
- The thymus confers immunological competence on the lymphocyte. In the thymus, prethymic lymphocytes are educated by hormone-like humoral factors—thymosin, thymopoietin, etc. produced by thymic epithelial cells, so that they become capable of mounting cell-mediated immune response (CMI)

2. Bursa of Fabricius

- It is a lymphoepithelial organ located near the cloaca in chickens
- It contains lymphoid follicles, which are differentiated into cortex and medulla and composed of immunoglobulin producing B cells
- Precursors of B cells (stem cells) from the yolk sac, fetal liver and bone marrow reach the bursa and mature into immunocompetent bursal lymphocytes or B cells capable of mounting humoral immune response. They exit through the peripheral blood and become seeded into the secondary lymphoid organs
- In mammals, equivalent of bursa has not yet been identified. The maturation of precursors of B cell into functionally mature B lymphocyte occurs in bone marrow and gut-associated lymphoid tissue

Functions

It is responsible for the development of immunocompetent B cells making humoral antibodies.

■ Describe peripheral lymphoid organs and also give their functions.

These are the organs which receive and maintain functional lymphocytes. The lymphocytes educated by central lymphoid organs are seeded into peripheral lymphoid organs where they initiate and participate in immune response to antigenic stimuli. The peripheral lymphoid organs include:

1. Lymph Nodes

- These are small round or oval bodies found in various parts of the body, located at major junctions of the network of lymphatic channels (Fig. 21.2)

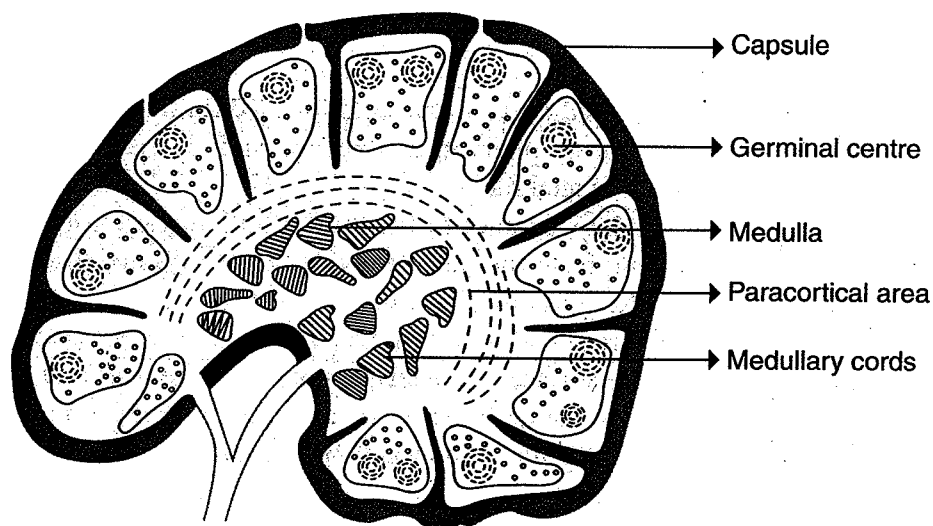


Fig. 21.2 Section of lymph node.

- The mature node is differentiated into outer cortex and inner medulla
- The cortex is subdivided into the external cortex located just below the capsule and a deep cortex also known as the paracortical area
- The external cortex contains primary lymphoid follicles (accumulation of lymphocytes) within which germinal centres (secondary follicles) develop following antigenic stimulation
- The follicles also contain dendritic macrophages, which capture and process the antigen
- In the medulla, lymphocytes are arranged as elongated branching bands (medullary cords). In activated lymph nodes, most of them are plasma cells secreting antibodies
- The cortical follicles and medullary cords contain B-lymphocytes (bursa-dependent area) while the T cells are occupied in paracortical area (thymus-dependent area)

Functions of Lymph Nodes

- Act as filters for foreign antigens
- Provide a site for phagocytosis and antibody production
- Support the development of lymphocytes

2. Spleen

- It is the largest lymphoid organ. The architecture is similar, but not identical to that of lymph node
- It contains two segregated areas—the red pulp and white pulp, separated by marginal zone
- The white pulp is rich in lymphoid tissue while the red pulp is abundant in sinuses and contains large number of RBCs
- The white pulp is located mainly around small arteries and the periarterial lymphatic sheath is composed primarily of T lymphocytes and is called the thymus-dependent area
- The external lymphoid area surrounding the periarterial lymphatic sheath is a B-dependent area (perifollicular region, germinal centre and mantle layer)
- The periarterial lymphoid collections in the white pulp are called Malpighian corpuscles or follicles. Following antigenic stimulation, germinal centres are produced in the white pulp that are composed of large numbers of rapidly dividing cells which differentiate into plasma cells and replace T cells in the periarterial region (Fig. 21.3)

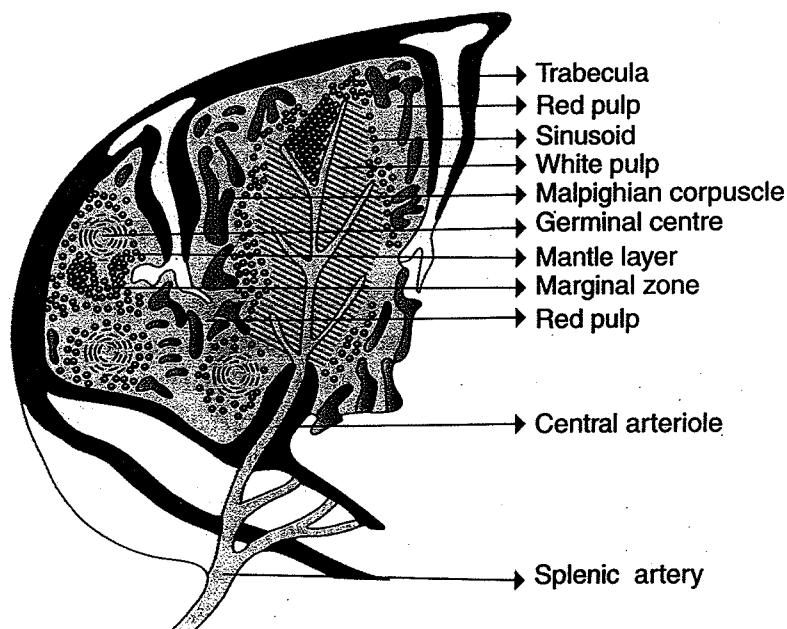


Fig. 21.3 Architecture of spleen.

Functions of Spleen

- It is a systemic filter for trapping circulating blood borne particles .
- It is a major site for antibody synthesis against blood borne particles; hence its immunological role is primarily directed against blood borne pathogens

3. Mucosa Associated Lymphoid Tissue (MALT)

- Lymphocytes producing IgA are present throughout the mucosal lining of alimentary, respiratory, genitourinary and other surfaces as isolated cells or small cell clusters, are called the MALT
- Such lymphoid tissues of the gut are known as gut associated lymphoid tissue (GALT) and those in the respiratory tract are called the bronchus associated lymphoid tissue (BALT)
- The main GALT structures in humans are:
 - Tonsils (lingual, palatine and pharyngeal)
 - Appendix (at the junction of small and large intestines)
 - Peyer's patches of the intestine
 - Lamina propria of the intestine
- The MALT structure contains mixture of B cells, T cells as well as phagocytic cells
- Secretory IgA is the main immunoglobulin produced by MALT. IgG, IgM and IgE are also produced locally

Functions of MALT

Local immunity against pathogens invading local tissue.

■ Name the important cells of the lymphoreticular system.

The cells of the lymphoreticular system consist of

- Structural cells—reticulum cells, endothelial cells and fibroblasts
- Immunocompetent cells—lymphocytes, plasma cells and macrophages. These cells play important role in immune response

■ What are lymphocytes? Describe their structure.**Lymphocytes**

- These are small, round cells found in peripheral blood, lymph, lymphoid organs and many other tissues
- In peripheral blood, they constitute 20–45% of the total leucocyte population and are predominant cell type in lymph and lymphoid organs

Structure of Lymphocytes

- Three types of lymphocytes occur—small, medium and large
- The small lymphocytes are necessary for immune response
- The properties of small lymphocytes are:
 - Size: 5–8 μ in diameter
 - Shape: Round
 - Nucleus: Spherical with prominent nuclear chromatin
 - Cytoplasm: A thin rim of cytoplasm with scattered ribosomes but devoid of endoplasmic reticulum, Golgi bodies and other organelles
 - Motility: Slowly motile. During movement assume a hand mirror-like form with nucleus in front and cytoplasm as a tail behind (Fig. 21.4)

■ Classify lymphocytes based on (a) Morphology, (b) Lifespan and (c) Functions.

Classification of lymphocytes:

(a) Based on Morphology

1. Small lymphocytes 5–8 μ
2. Medium lymphocytes 8–12 μ
3. Large lymphocytes 12–15 μ

(b) Based on Lifespan

1. Short-lived/effector cells, 2 weeks—immune response
2. Long-lived/memory cells, 3 years or more—storehouse of immunological memory

(c) Based on Function

1. Antibody producing B cells
2. CMI producing T cells

The T and B lymphocytes are two groups of nonphagocytic, morphologically indistinguishable cells which are functionally different but have certain common features:

- They re-circulate throughout the body. There is constant circulation of lymphocytes through the blood, lymph, lymphatic organs and tissues. So that when an antigen enters into any part of the body, lymphocytes of appropriate specificity would reach the site and mount an immune response. Re-circulating lymphocytes can be recruited by the lymphoid tissue whenever necessary. Majority of the re-circulating cells are T cells. B cells tend to be more sessile
- Each lymphocyte possesses specific receptor on its surface for recognition of a particular antigen. This confers specificity
- When stimulated by specific Ag, the lymphocytes undergo clonal proliferation B cells are transformed into plasma cells, which synthesize antibodies while T cells produce lymphokines and induce CMI
- They show the property of memory, giving rise to a faster and powerful immune response on re-challenge with the same antigen

■ Differentiate between T and B lymphocytes.

The lymphocytes are differentiated into 'T' and 'B' based on their surface markers (Table 21.1). The various tests used in differentiation of T and B cells are:

1. T cells bind to sheep erythrocyte at 37°C and form SRBC rosettes by CD2 antigen while B cells do not
2. B cells bind to sheep erythrocyte coated with IgG and complement and forms EAC (erythrocyte Ab complement) rosettes. EAC rosette formation is due to the presence of a C3 receptor on B cell surface. T cells do not form EAC rosette
3. T cells have thymus specific Ags on their surface which are absent on B cells
4. B cells have Igs on their surface. They also possess surface receptors for the Fc fragment of IgG while T cells possess the TCR (T cell receptor) linked to CD3 for recognition of Ag
5. The T cells undergo blast transformation on treatment with mitogens such as phytohaemagglutinin (PHA) or concanavalin-A (Con-A), while B cells undergo similar transformation with bacterial endotoxins

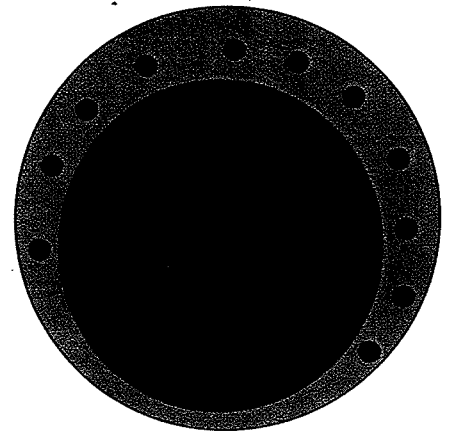


Fig. 21.4 Structure of a lymphocyte.

Table 21.1 Distinguishing characteristics of T and B cell

Character	T Cell	B Cell
1. CD3 receptor	+	-
2. Surface immunoglobulins	-	+
3. Receptor for Fc portion of IgG	-	+
4. EAC rosette	-	+
5. SRBC rosette	+	-
6. Thymus specific antigens	+	-
7. Numerous microvilli on surface	-	+
8. Blast transformation with:		
(a) Phytohaemagglutinin	+	-
(b) Concanavalin A	+	-
(c) Bacterial endotoxins	-	+
(d) Anti-CD3	+	-
(e) Anti-Ig	-	+

CD3 = cluster of differentiation 3, EAC = erythrocyte Ab complement, Ig = immunoglobulin, IgG = immunoglobulin G, SRBC = sheep RBC.

6. When viewed under scanning microscope, B cells show numerous villi-like projections (microvilli), while T cells have few or no cytoplasmic villi-like surface projections

■ List the general functions of lymphocytes.

The lymphocytes educated by the central lymphoid organs become immunologically competent cells (ICC) and subserve the following general functions:

- Recognition of Ag and storage of immunological memory
- Immune response to specific Ag and resistance to certain infections
- Transplantation and tumour immunity
- Hypersensitivity

■ Describe the various types of T lymphocytes.

T cells originate from precursor cells from bone marrow and migrate to the thymus and mature there. Several subsets of the T cells arise during the maturation process; each one is responsible for specific function. The different subsets are:

T-helper (CD4) Cells (Th)

- Constitute 55–70% of the total T cells
- Two subsets—Th1 and Th2. Th2 are the principal helper cells
- Interact with B cell, promote proliferation of B cells and enhance Ab synthesis
- They also enhance activation of macrophages and differentiation of cytotoxic T cells

T-suppressor (CD8) Cells (Ts)

- Constitute 25–40% of circulating T lymphocytes
- They block antibody production by acting on Th cells or by acting directly on B cells by secreting suppressor substances

T-regulator Cells (Tr)

- Tr cells are the regulator cells, which regulate the activity of Th and Ts cells

Cytotoxic (Tc) T cells (CD8 Cells)

- These cells are responsible for killing of specific target cells whose surface antigens can be recognized by them, e.g. virally infected host cells, tumour cells and allogenic graft cells in transplanted tissues
- They possess membrane glycoprotein—CD8 receptors on their surface
- They cause destruction of target cells by releasing lymphotoxins and perforin

Delayed Hypersensitivity (DTH) T Cells (TD Cells)

- Responsible for delayed type of hypersensitivity reactions
- They are indistinguishable from Th-cells on the basis of surface markers
- They possess CD4 markers
- They secrete different lymphokines, which are responsible for inflammatory response of delayed hypersensitivity and growth factors, which are believed to regulate lymphocyte activity

■ Describe B lymphocytes.

B cells originate from precursor cells of the bone marrow. During the maturation process, the pre-B cell is programmed to produce only one class or subclass of Ig after a switch from initial IgM production. On the basis of immunoglobulin which is programmed to synthesize, B lymphocytes can be subdivided into different subsets—IgM, IgG, IgA, IgD and IgE.

Activation of B cells

Ig present on the surface of B cell acts as the specific receptor for Ag. When Ag enters into the body, it reacts with B cell with appropriate specificity. This interaction stimulates B cell to undergo blast transformation and convert it into plasma cell.

Each B cell possesses information to produce Ab of unique Ag specificity as a membrane receptor. Once the signal is received, B cells are differentiated into plasma cells, which produce and secrete Abs.

■ Enumerate the properties and functions of plasma cells.

Antigenically stimulated B cells undergo blast transformation to form plasma cells. The properties of mature plasma cells are:

- Shape: Oval
- Size: Twice the size of small lymphocyte
- Nucleus: Small, eccentric, oval with radially arranged chromatin around the periphery that gives the appearance of a clock face or cartwheel
- Cytoplasm: Large, contains a well-developed Golgi apparatus and abundant endoplasmic reticulum
- Lifespan: These are end cells and have a short lifespan of two or three days

Function

Plasma cell is an antibody-producing machinery. It can produce an Ab of a single specificity—IgM, IgG, IgA, IgD or IgE.

■ What are null cells? Describe the various types of null cells.

A small proportion of lymphocytes (about 5% to 10%), which cannot be classified as either T or B, are known as null cells.

The null cells are of three types:

1. Natural Killer Cells (NK Cells)

- They are so called because they are found in normal animal
- They are also known as large granular lymphocytes (LGL), as they are nearly twice the size of small lymphocytes. Cytoplasm contains several azurophilic granules composed of mitochondria, ribosomes, endoplasmic reticulum and Golgi apparatus. They are present naturally and are not formed in response to Ag
- They can kill a variety of transformed cells, virally infected host cells and are also involved in allograft rejection
- NK cells are present in the spleen and peripheral blood
- They do not require Ab and their action is nonspecific. Their activity is increased by interferon and IL-2
- The lysis of cell is because of release of several cytolytic factors. These are the glycoproteins, which contribute to cytotoxicity. These include:
 - Perforins—resemble complement component C9, cause transmembrane pores through which the cytotoxic factors such as TNF enter the cell and destroy it by apoptosis (programmed cell death)
 - Lymphotoxin (LT)
 - Tumour necrosis factor (TNF)
 - Natural killer cytotoxic factor (NKCF)

2. Killer Cells (K Cells)

- These are lymphocytes possessing receptors for the Fc portion of IgG
- They are able to lyse or kill target cells sensitized with IgG Abs
- They mediate Ab dependent cell cytotoxicity (ADCC) in which an Ab molecule forms a bridge between the target cell and K cell. The Fab portion of IgG binds with the antigenic determinants on the target cell and Fc portion of the IgG with Fc receptor on the K cells and damage to target cell without the help of complement

3. Lymphokine-activated Killer Cells (LAK Cells)

These are NK cells treated with interleukin-2 (IL-2), which are cytotoxic to tumour cells. They can be used in the treatment of some tumours such as renal cell carcinoma.

■ What are phagocytic cells?

Cells that are responsible for inactivation, removal and disposal of microorganisms, often with the help of Ab and complement are called phagocytic cells. Phagocytic cells were first described by Metchnikoff (1883).

■ Write a short note on each of the following types of phagocytic cells: Macrophages, Microphages.

Macrophages

- These are large round or oval cells with kidney or oval-shaped nucleus and abundant cytoplasm
- The blood macrophages (monocytes) are 12–15 μ in size while tissue macrophages (histiocytes) are 15–20 μ in size
- They are widely distributed throughout the body
- They are produced from the stem cells in the bone marrow where they undergo proliferation and are delivered as monocytes in the bloodstream, after a period of maturation (about 6 days)
- The blood monocytes, after few days, migrate to various tissues where they differentiate into macrophages

- Monocytes in blood have half-life of three days while macrophages in tissue survive for months by proliferating locally

Microphages

- These are small nondividing polymorphonuclear leucocytes or granulocytes present in blood
- Three types—neutrophils (45–60%), eosinophils (1–3%) and basophils (0.3%)
- The neutrophils, and to a lesser extent the eosinophils, are phagocytic
- They contain granules and a wide range of bactericidal substances
- They originate in the bone marrow from stem cells, undergo maturation and finally released into the circulation
- They are short-lived cells with half-life of 2 days in circulation and few hours in tissue after penetration

■ Enumerate the functions of phagocytic cells.

Functions of phagocytic cells are:

- The primary role is the phagocytosis—engulfment and digestion of foreign particles
- Also participate in the development of specific immune response, e.g. trapping of Ag by macrophages and its presentation to lymphocytes in optimal concentration
- Macrophages also participate in antitumour activity and graft rejection
- Microphages participate in inflammation, opsonization, hypersensitivity reactions and immunity against parasitic infections

■ Define phagocytosis.

Engulfment and digestion of foreign particles by a single cell is known as phagocytosis. It is the most important means of nonspecific defense mechanism against microorganisms.

■ Describe the process of phagocytosis.

Phagocytosis occurs in the following steps:

1. Chemotaxis

- The entry of foreign particles such as microorganisms causes release of chemotactic substances derived from the complement system or lymphocyte-derived factors
- Microorganisms react with their Abs and then with C (complement). Some of the components of C diffuse out from the centre of reaction and establish a concentration gradient of chemotactic factors (C3a, C5), which attract microphages
- The sensitized lymphocytes stimulated by specific Ag produce lymphokines such as macrophage chemotactic factor or macrophage-activating factor that are chemotactic for monocytes and macrophages
- This causes aggregation of phagocytic cells at the site of infection

2. Adherence (Attachment)

The phagocytic cell attaches to the infective agent through specific receptors.

3. Ingestion and Digestion

Once the contact is made with a foreign particle, engulfment starts with a deep invagination of the cell membrane, which fuses to form a pouch called **phagosome**. Phagosome is transported deep into cytoplasm where it fuses with lysosome to form **phagolysosome**. The lysosome ruptures and releases its enzymatic contents, which come in contact with the ingested particle. A battery of mechanisms slaughters the ingested particle. Lysosome contains a variety of hydrolytic enzymes

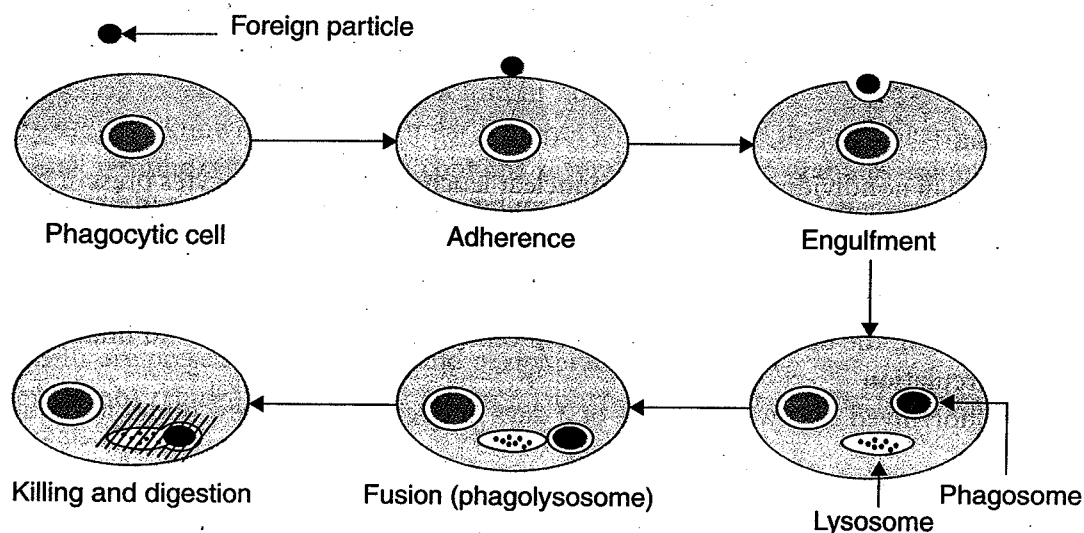


Fig. 21.5 Process of phagocytosis.

such as glucuronidases, lipases, nucleases, peroxidases, phosphatases, lysozyme, phagocytin and other bactericidal substances, which quickly, within 15 minutes, kill most of the microorganisms (Fig. 21.5).

■ Describe dendritic cells.

- These are the Ag presenting cells (APC)
- They are derived from bone marrow and are different from the macrophages and T and B lymphocytes
- They have little or no phagocytic activity
- They are highly pleomorphic
- They are present in peripheral blood and in the peripheral lymphoid organs, especially in the germinal centres of the spleen and lymph nodes
- They play an important role in the presentation of antigens to T cells during the primary immune response
- Ag capture by the dendritic cells of the lymph node follicles occurs in the presence of pre-existing Ab

■ What is major histocompatibility complex (MHC)?

- The major histocompatibility complex (MHC) is a region on chromosome consisting of closely linked cluster of genes that code for a number of cell surface and plasma protein antigens
- The MHC in mammals also consists of a region where the histocompatibility-linked immune response genes (IR) are located. This chromosomal segment controls:
 - Synthesis of transplantation antigens and graft rejection
 - Immune response to infection
 - Susceptibility to the development of immunologically-mediated diseases
- The two MHC systems that have been most extensively studied are:
 - The H2 antigen system in the mouse
 - The HLA (human leucocyte antigen) system in human beings
- The major antigens determining histocompatibility in human beings are alloantigens found on the surface of leucocytes, hence they are known as HLA, and the MHC system is known as the HLA system

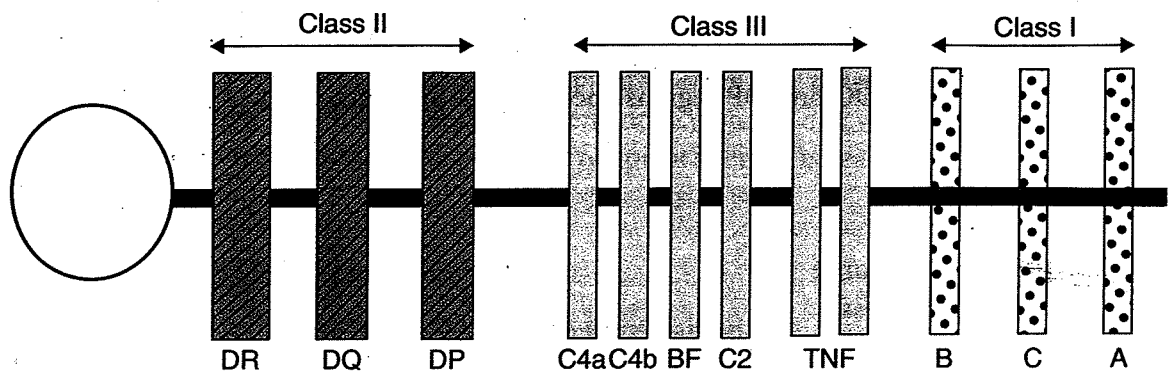


Fig. 21.6 HLA complex.

■ Describe HLA complex?

- The histocompatibility Ags are alloantigens present on the surface of leucocytes in human beings and are called human leucocyte Ags
- The set of genes coding for HLA Ags is known as HLA complex
- The HLA complex of genes is located on the short arm of autosomal chromosome—6
- It is divided into three separate clusters of genes, each of which codes for one of the major classes of antigens
 - Class I—A, B and C loci
 - Class II or the D region—DR, DQ and DP loci
 - Class III or the complement region—C2 and C4 of the classical pathway, properdin factor of the alternate pathway and tumour necrosis factor α and β (Fig. 21.6)
- The HLA system is highly pleomorphic. Pleomorphism is very high in class I and class III antigens. For example, there are at least 24 alleles at HLA-A locus and 50 at HLA-B locus. Each allele determines a distinct Ag. Every individual inherits one set of HLA genes from each parent

■ State important features and functions of histocompatibility antigens.

The important features and functions of histocompatible antigens, namely class I, class II and class III antigens are described below:

1. Class I Antigens

- The class I antigen is a transmembrane (α -chain) glycoprotein with molecular weight 44 kd
- It is noncovalently associated with β -2 macroglobulin (β -chain) of molecular weight 12 kd
- The α -chain is encoded by three structural genes in the HLA-A, B and C regions and its products are the HLA-A, B and C antigens
- The MHC-class I antigens are found on the surface of most nucleated cells and occur most abundantly on lymphoid cells

Functions

- Essential for immune T-cell recognition of specific target antigens. Lymphocytes can only bind to antigens that are associated with these molecules
- They are involved in graft rejection and elimination of virus-infected host cells (cell-mediated cytotoxicity)
- The CD8 cells are specific for MHC-class I antigens

2. Class II Antigens

- These are heterodimers consisting of two noncovalently bound glycoprotein chains called α - and β -chains of about 34 kd and 29 kd molecular weight respectively

- The class II antigens are distributed on limited cell types and are found on macrophages, monocytes, dendritic cells, activated T lymphocytes (CD4) and B lymphocytes
- Genes of HLA—DR, DQ and DP regions encode these antigens

Functions

They play a major role in

- Graft versus host response
- Immune responsiveness
- Immune suppression
- Cellular recognition
- Cellular interactions
- Mixed lymphocyte reaction

3. Class III Antigens

- These are heterogenous molecules
- Include complement components C2, C4 and factor B (components responsible for the formation of C3 convertase)
- They also include heat shock proteins and tumour necrosis factor
- These molecules are encoded by class III MHC genes

Functions

As they include complement components, they are concerned with complement activation.

■ Explain HLA typing.

The HLA typing is most important in tissue typing and tissue matching in transplantation and in paternity determination, anthropological surveys and in establishment of association between HLA and disease susceptibility. It is done by the following methods:

1. Detection of Class I Antigens

HLA A, B and C are identified by complement dependent cytotoxic reactions using monospecific antisera obtained from patients with whole blood transfusion and multiparous women (women who have had multiple pregnancies) who are often sensitized by foetal antigens. Monoclonal antibodies to antigens are now available.

- Donor lymphocytes are typed against the panel of such standard and recipient sera in the presence of complement
- Observed for cell death. Inability of cell to exclude trypan blue or eosin indicates its death
- Based on cell death phenotype of an individual is identified

2. Detection of Class II Antigens

The class II antigens are identified by mixed leucocyte reactions (MLR).

In this test, cells of two nonidentical individuals (donor and recipient) are mixed together. One cell, which is the stimulator, is irradiated, and it does not proliferate. The other cell, the responder, recognizes class II antigens (foreign) on the stimulator cells and begins to proliferate. This proliferation is measured by the uptake of H3 thymidine into new DNA over a period of 5 or 6 days. The amount of new DNA indicates the degree of foreignness. More the DNA synthesis more is the foreignness.

3. Detection of Preformed Cytotoxic Antibodies

The preformed cytotoxic antibodies in recipient's serum are detected by mixing recipient's serum with donor's lymphocyte and complement. Cytotoxic or cytolytic effect of recipient's serum on donor's lymphocytes indicates that recipient's serum is reactive against the graft.

Indications of HLA Typing

The indications of HLA typing are:

- Tissue typing and tissue matching prior to transplantation
- Paternity determination
- To establish association between HLA and disease susceptibility

■ **Write a short note on MHC restriction.**

- T cells respond to antigens on the macrophages only when they are presented along with the self-MHC antigen. This is known as MHC restriction
- When antigen enters, it is processed and degraded by macrophages and the processed antigen that appears on the surface of the macrophage is presented to T cells to initiate an immune response
- The immune response is initiated when both cells possess same MHC antigen This is because of MHC restriction
- Both class I and class II MHC antigens interact with macrophages or target cells
- Cytotoxic T cells (CD8) react with antigen in association with class I MHC antigens while helper T cells (CD4) recognize the class II MHC antigens on the surface
- Cytotoxic T cells are able to interact and kill or lyse virally infected target cells only when the T cells and target cells are of the same MHC type

22

Chapter

The Immune Response

■ Explain the immune response.

The immune response involves:

- Recognition of the foreign Ag, and
- Mounting of an immunological response for elimination of an Ag

Immune response is of two types:

1. The humoral or Ab-mediated immune response
2. The cellular or cell-mediated immune response

These two are usually developed together, though at times either of the two responses become predominant or any one may develop exclusively.

■ Comment on the fate of immunogens in human body.

Entry of Ag in the body may occur naturally or artificially by various routes. When injected intravenously, it rapidly localizes in the spleen, liver, kidneys, bone marrow and lungs. When injected intradermally or subcutaneously; the major portion of the Ag remains at the site of injection or is localized in the draining lymph nodes. The physicochemical properties determine the degree of localization in the lymph node, e.g. particulate Ags such as viruses are more effectively retained in the lymph node than soluble Ags. Ags that enter by ingestion and inhalation are localized in lymphoid tissue of gastrointestinal and respiratory tracts and initiate an immune response. Most Ags are readily taken up by phagocytic cells (macrophages) or dendritic cells. These are the Ag presenting cells, which process and present Ag to immunocompetent lymphocytes to initiate the immune response.

■ Discuss the Ab-mediated (humoral) immune response to (a) T-dependent antigens and (b) T-independent antigens.

(a) Ab-mediated (Humoral) Immune Response to T-dependent Ags

Abs are produced by B lymphocytes but some Ags such as RBCs, serum proteins and a variety of protein-hapten conjugates require help of T cells and are known as T-dependent Ags. Majority of the Ags are T-dependent Ags.

When T-dependent Ags are injected repeatedly, there is increase in Ab response and also shifting of type of Ab response from IgM to IgG. This is due to T-cells and T-dependent Ags. The shifting Ab responses elicited by T-dependent Ags are especially evident when the initial (primary) and subsequent (secondary) responses to the antigenic stimuli are compared.

Primary Immune Response

- Immune response which develops when Ag enters the body for the first time (priming dose), is called the primary immune response (Fig. 22.1)

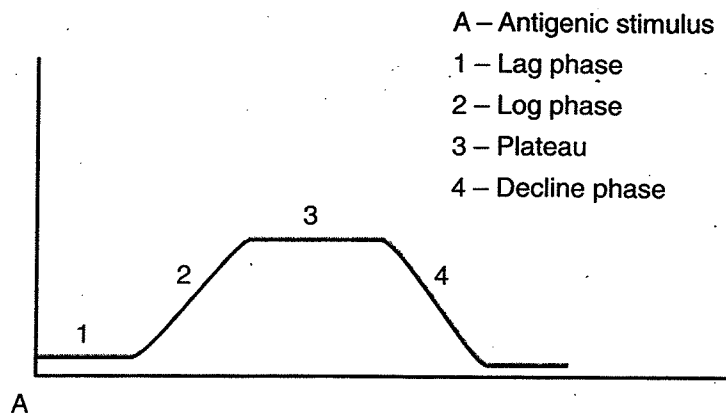


Fig. 22.1 Primary immune response.

- The primary response is
 - S—slow
 - S—sluggish
 - S—short-lived
 - Has long lag phase—5 to 7 days
 - Shows low titres of Abs that persist for short duration
 - Produces IgM type of Ab

Secondary Immune Response

- When the same Ag enters for the second time (booster dose), after weeks, months or even after years, the response produced by the body is called the secondary immune response (Fig. 22.2)
- Following the booster dose, there is a markedly enhanced response that is characterized by the accelerated appearance of immunocompetent cells and Ab
- The secondary response (anamnestic or recall) is
 - P—prompt
 - P—powerful
 - P—prolonged
 - Has short or negligible latent phase (2 or 3 days)
 - Produces high levels of Abs—lasting for long period
 - Produces IgG type of Ab

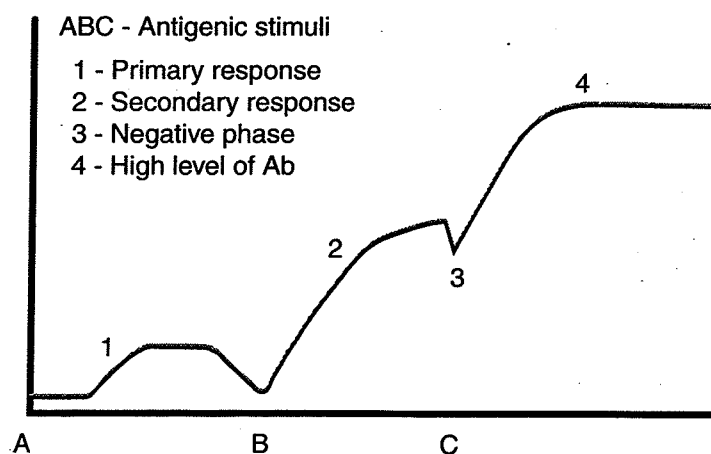


Fig. 22.2 Secondary-immune response.

- If the specific Ab is present in the serum at the time of booster dose, the Ab disappears rapidly. This is the negative phase, which is due to the combination of the Ab with newly injected Ag
- The differences in primary and secondary response are due to the number of responding B cells
- In the primary response, only few B cells are converted into Ab-producing B cells and majority of B cells are converted into memory cells. Memory cells are long lasting cells, which are able to respond to the same Ag when it enters for the second time and help the B cells to produce Abs in high titre. The life of memory cells is 3 years or more
- In the secondary response, all B cells are converted into Ab-producing B cells

Role of Th Cell

- When Ag enters, Th cells recognize carrier molecule and the hapten reacts with B cell, but as hapten is unable to stimulate Ab synthesis, B cells are not stimulated
- When Th cell with carrier molecule cooperates with B cells and provides necessary signal, B cells undergo proliferation to form plasma cells

Role of Ts Cells

- Ag can also stimulate Ts cells, which suppress Ab production by blocking T-helper cells or perhaps by acting directly on B cells
- It has been suggested that Ag bound to macrophage stimulates Th cells whereas Ag, which is not bound to macrophage stimulates Ts cells

Synthesis and Secretion of Ig

Once the proper signal is noticed, B cells undergo clonal proliferation and blast transformation, and are finally converted into plasma cells that synthesizes and secrete Ab.

Like other proteins, immunoglobulins are synthesized on ribosomes attached to endoplasmic reticulum. Then the precursor enters into Golgi bodies and finally towards the cell surface in a secreting vesicle. The vesicle fuses with the surface membrane and releases Ig molecules. During this migration the completed chains are assembled, the interchain disulphide bonds are formed and sugars are added.

(b) Ab-mediated (Humoral) Immune Response to T-independent Ags

Some Ags, e.g. bacterial lipopolysaccharides, pneumococcal polysaccharides, polymerized flagellin, ferritin, etc. have multiple repeating epitopes (antigenic determinants) on their surface naturally, and hence do not require processing by T cells. These antigens are known as thymus-independent antigens, which activate B cells directly without the help of T cells. These antigens elicit production of IgM antibodies only as memory is lacking. The response to second injection is also same as that of first. It means that primary and secondary responses to these Ags are same.

■ Mention the scope of humoral immunity (HI).

Humoral immunity is important in the following ways:

- Primary defense against most extracellular pathogens
- Mediate defense against viruses infecting through respiratory and intestinal tracts
- Prevents recurrence of virus infections
- Participates in immediate type of hypersensitivity
- Participates in the pathogenesis of certain autoimmune diseases

■ What are monoclonal antibodies?

Milstein and Kohler (1975) devised a technique for the production of immortal clones of hybrid cells, making Ab of single specificity by fusing normal Ab-producing lymphocytes with mouse myeloma cells. These clones are termed hybridomas.

The hybridoma produces antibodies specifically directed against a single antigen or antigenic determinant. Such antibodies produced by a single antibody-forming cell or clone and directed against a single antigen or antigenic determinant are called monoclonal antibodies.

■ **With the help of suitable illustrations, describe the technique of hybridoma production.**

Production of hybridoma involves the following steps:

- Mouse is immunized with a desired Ag and B lymphocytes are harvested from the spleen
- B cells from spleen are fused with mouse myeloma cells grown in a tissue culture and are placed in hypoxanthine aminopterin thymidine (HAT) medium for 10–14 days. As myeloma cells lack hypoxanthine phosphoribosyl transferase, they die in HAT medium. Normal lymphocytes die as they have short lifespan and they cannot replicate indefinitely. Only hybrid cells possessing properties of both cells can grow in the medium. These are called hybridomas
- The hybridomas are cloned and examined for production of antibodies and clones producing desired Ab are selected and grown in bulk for production of monoclonal Abs
- Hybridomas can be maintained in culture indefinitely and used for production of monoclonal antibodies. They can also be grown as tumours in the peritoneal cavity of mice by injecting intraperitoneally and monoclonal antibodies obtained by harvesting the ascitic fluid (Fig. 22.3)
- Hybridomas can also be stored in a frozen state for future use

■ **Write down the applications of monoclonal Abs.**

Monoclonal Abs have the following applications:

- Diagnostic use—for diagnosis of microbial infections, cancer, autoimmune diseases, for serotyping of microorganisms (identification), etc.
- Treatment of cancer
- Vaccine preparation—to purify the antigens
- To generate pure antibodies from impure antigen

■ **List the factors affecting Ab production and highlight their important features.**

Factors affecting Ab production are:

1. Age

- The embryo is immunologically immature
- The Ab production starts with the development and differentiation of lymphoid organs
- During embryonic life, when Ag comes in contact with immunocompetent cell, it results into permanent inactivation or death of the cell (induction of tolerance). This is believed to be the basis for the nonantigenicity of self-antigens
- Immunocompetence is not complete at birth but it develops slowly as the infant grows and the full competence is acquired by the age of 4 years

2. Nutritional Status

- Protein calorie malnutrition suppresses both humoral and cell-mediated immune response
- Deficiencies of amino acids such as tryptophan, phenyl alanine, methionine, glycine, isoleucine and vitamins such as vitamin A and B have been shown to cause decrease in Ab synthesis

3. Effect of Ab

- Animals, whose blood contains preformed Abs, which are present because of passive transfer or passive immunization, respond poorly to homologous Ag. These Abs combine with Ag and prevent their contact with immunocompetent cells

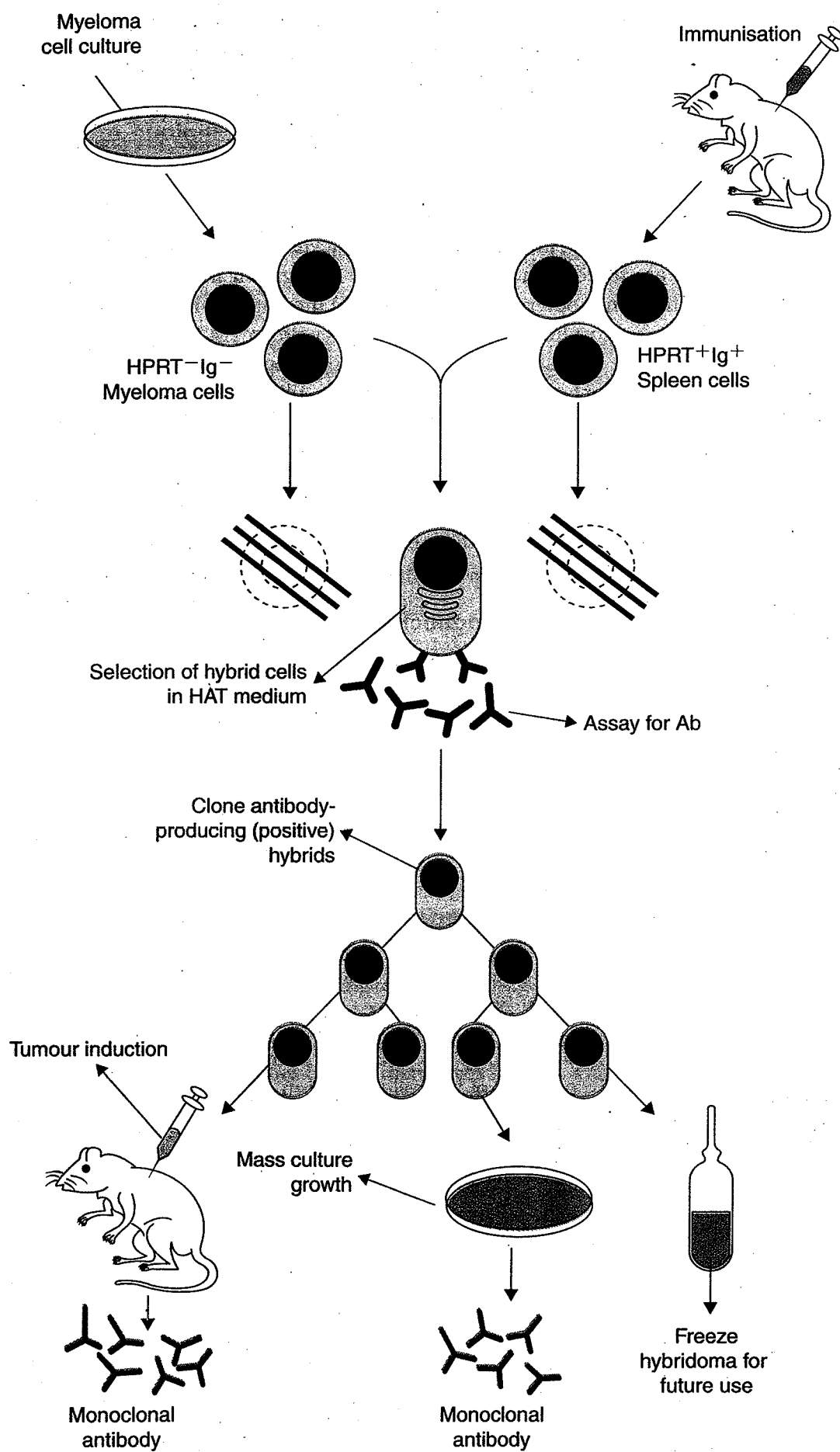


Fig. 22.3 Hybridoma technique.

4. Route of Administration

- The parenteral route is better for the development of HI than oral or nasal route
- For large particulate Ags such as bacteria or RBCs, intravenous route is more effective. However, soluble antigens are more effective when injected in tissues

5. Dose of Ag

- An Ag is effective only above a minimal critical dose. Very small amounts of Ag do not induce demonstrable Ab response
- Very large dose of Ag also (beyond a level) does not increase Ab production. In fact, very large doses of Ag may inhibit Ab production (immunological paralysis)

6. Multiple Ags

- The simultaneous administration of two or more Ags may result in enhanced Ab response to one or the other Ags or diminished Ab response to one or the other of them
- When two bacterial vaccines (e.g. typhoid and cholera) are given together, the Ab response to each is not influenced by the other
- When toxoids are given along with bacterial vaccines (e.g. DPT—diphtheria toxoid, pertussis vaccine, tetanus toxoid), the Ab response to toxoid is potentiated but when one of them is given in excess, the response to the other is diminished

7. Immunosuppressive Agents

- The synthesis of Abs can be inhibited by the administration of various immunosuppressive agents
- These include X-irradiation, cortisone, purine and pyrimidine analogues, nitrogen mustards, actinomycin D, cyclophosphamide and folic acid antagonists such as methopterin, and methotrexate
- Analogues of purine are 6-mercaptopurine and azathioprine and analogue of uracil is 5-fluorouracil and of cytosine is cytosine arabinoside
- Antilymphocyte serum (ALS) raised against lymphocytes and thymocytes in horses is also used as an immunosuppressive agent
- Cyclosporine is the most widely used agent, which is not cytotoxic for lymphocytes and has no antimitotic activity. It selectively inhibits T-helper activity

8. Adjuvants

- These are the substances which, when injected together with Ag, enhance immune response by sustained release of Ag (releasing Ag slowly) from injection site and increase the contact period of Ag with macrophage or other Ag receptive cells. This is known as 'Depot' effect of the adjuvant
- The adjuvant preparations suitable for human use are:
 - Aluminium adjuvants—potassium aluminium sulphate (alum), aluminium hydroxide, aluminium oxide
 - Freund's incomplete adjuvant (1940)—water in oil emulsion
 - Anaerobic coryneform—*Propionibacterium acnes*
 - BCG (Bacille Calmette-Guérin)
 - *Bordetella pertussis* (DPT)
- The adjuvant preparations suitable for laboratory animals are:
 - Freund's complete adjuvant—water in oil emulsion with mycobacterial or nocardial Ag
 - Bacterial endotoxin
 - Lysolecithin analogues
 - Vitamin A in toxic dose
 - Fungal polysaccharides

■ Discuss the theories of Ab production.

- Two types of theories have been proposed to explain the mechanism of Ab production. These are:
 - Instructive theories
 - Selective theories
- According to instructive theories, an immunocompetent cell (ICC) is able to synthesize Ab of any specificity. The Ag instructs ICC to produce the complementary Ab. However, according to selective theory, ICC have only restricted immunological range. The Ag selects and stimulates the appropriate ICC to synthesize an antibody. In addition to these theories, **side chain theory** was proposed by Ehrlich in 1900

Side chain theory

- It is the first theory and was proposed by Ehrlich (1900)
- According to this theory, the cells have side chains, which are normally used for the assimilation of nutrients
- When Ags are introduced, they combine with those side chains, which are complementary fit-in (receptors) and inactivate the receptors, and interfere with absorption of nutrients
- Hence, cell produces same type of receptors to keep the cellular metabolic pathway open
- These receptors spill over into blood and circulate as Abs
- This theory is not accepted as it fails to explain secondary response, immunological tolerance and autoimmune diseases

Instructive Theories

1. Direct Template Theory (Ag Template Theory)

- According to this theory, the Ab is synthesized as a normal globulin and is then brought into contact with the Ag, during this contact an Ag acts as a template for arrangement of polypeptide chain to form Ab molecule
- This theory is not accepted

2. Indirect Template Theory

- Proposed by Burnet and Fenner (1941)
- According to this theory, the antigenic determinant produces heritable changes in the Ab producing cell, so that a 'genocopy' of the antigenic determinant is incorporated in its genome and transmitted to progeny cells (indirect template)
- This theory explains specificity, secondary response and nonantigenicity of self-Ags

Selective Theories

An alternative view holds that the information necessary for different antibodies is already present in genetic material of Ab-producing cell. When antigen reacts with Ab-producing cell, the gene, which codes for a specific Ab is selected and switched on and Ig chains are synthesized through transcription and translation, which fold spontaneously to form an Ab molecule in absence of Ag.

1. Natural Selection Theory (Jerne's Theory)

- Proposed by Jerne (1955), this theory postulates that millions of globulins (Abs) are formed during embryonic life, which cover the full range of antigenic specificity
- These globulins act as specific receptor molecules, and when Ag is administered, it selectively combines with specific receptor molecule, which is complementary fit and forms a complex
- This complex is then homed in on the Ab-producing cell and stimulates them to produce the same type of Ab

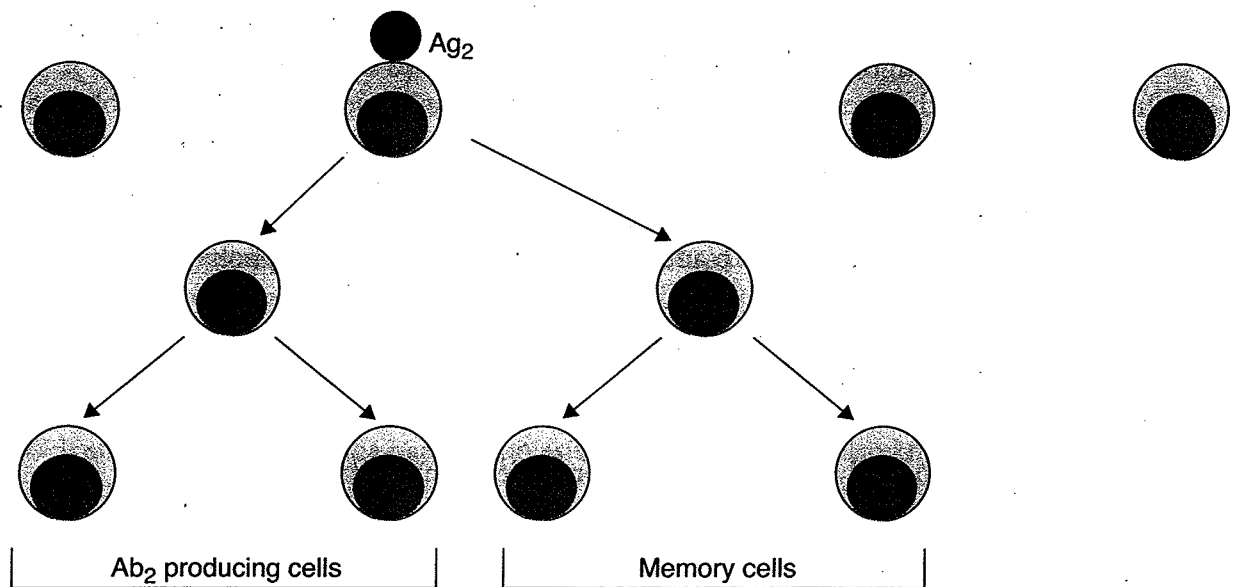


Fig. 22.4 Clonal selection model.

- This theory is also not acceptable as it postulates the selection at the level of Ab molecule and it does not explain the fact that immunological memory resides in the cells and not in serum

2. Clonal Selection Theory

- It is the most widely accepted selective theory proposed by Burnet (1957)
- According to this theory, the Ig molecules are present on the surface of B cells as specific receptors for a specific Ag
- When Ag is introduced, it combines with that Ig molecule on the cell surface, which is complementary fit for it
- This interaction results in proliferation of only those lymphocytes, which possess specific Ab molecules on their surface, to form a clone of cells producing Ab of same specificity as that on the surface of parent lymphocyte
- Some of the progeny cells are converted into memory cells
- Here, an Ag selects a specific B cell and stimulates it to proliferate into a clone of cells producing specific Ab (Fig. 22.4)

■ Explain the cell-mediated immune response (cell-mediated immunity).

Cell-mediated immunity (CMI) is the specific acquired immune response mediated by sensitized T cells. Certain microorganisms such as:

- Bacteria—Tubercle and leprosy bacilli
- Viruses—Smallpox and measles
- Parasites—*Toxoplasma* and *Leishmania*
- Fungi—*Histoplasma* and *Blastomyces*

These organisms are intracellular pathogens, which multiply inside the host cells. Hence, Abs are not effective against such pathogens. Immunity against these pathogens is mediated by the T lymphocytes. Such an immune response, which involves the interaction of cells of the immune system with the Ag, is known as CMI.

■ How is induction of CMI brought about?

- The nature of antigenic stimulus is important in the induction of CMI. The intracellular parasites are the best stimulators

- The T cells possess specific receptors (TCR) on their surface for Ag
- The binding of Ag with this receptor on T lymphocyte initiates CMI
- When Ag reacts with T cells, they undergo proliferation and a sequence of morphological and biochemical events occur and the cell transforms into a large blast cell—the process is called blast transformation
- This interaction finally results into blast transformation, clonal proliferation and differentiation resulting in the generation of Th and Ts cells, cytotoxic T cells, lymphokines-producing T cells and memory cells

■ Name two major effector mechanisms of CMI responses.

Proliferated T lymphocytes provide two major effector mechanisms:

1. The release of biologically active soluble factors called lymphokines (cytokines)
2. The generation of cytotoxic T cells

■ What are cytokines?

Cytokines

Cytokines are biologically active substances released by cells of immune system. These include:

Lymphokines

These are biologically active substances released by activated T lymphocytes. These are regulatory proteins with molecular weight 20,000–80,000, whose main function is to regulate immune response and growth and function of cells of reticuloendothelial system. They have several biological activities.

Monokines

These are soluble proteins produced by monocytes or macrophages. They are responsible for various biological effects.

The lymphokines, monokines, interferons, growth factors and other factors have similar effects. Hence, they are grouped as cytokines.

Interleukins

The term interleukin is introduced for those products of leucocytes, which exert regulatory influence on other cells.

Most cytokines exhibit multiple biological effects and the same effect may be caused by different cytokines. Hence, the term interleukins is used collectively for the products secreted by leucocytes.

■ Describe the biological activities of cytokines.

The cytokines have several biological activities. These are as follows:

1. Cytokines Affecting Lymphocytes

A. Interleukin-1 (IL-1)

- It is a stable polypeptide—a monokine secreted by macrophages and other Ag presenting cells. It occurs in two molecular forms—IL-1 α and IL-1 β
- Its production is stimulated by Ags, toxins, injury and inflammatory processes and is inhibited by cyclosporine-A and corticosteroids
- Immunological effects of IL-1 are:
 - Stimulates Th cells for the production of IL-2 and other lymphokines
 - Stimulates B cell proliferation and Ab synthesis
 - Stimulates neutrophil chemotaxis and phagocytosis
- It has beneficial effect in severe infections in immunocompromised hosts

B. Interleukin-2 (IL-2)

- It is a cytokine produced by activated T-cells
- It is a T cell growth factor that induces proliferation of T-cells
- It is a powerful modulator of the immune response
- It promotes growth and differentiation of T and B cells and stimulates cytotoxic T and NK cells
- It converts large granular lymphocytes into lymphokine-activated killer (LAK) cells, which can destroy NK-resistant tumour cells. This property can be used in the treatment of certain types of cancers
- It stimulates secretion of other lymphokines

C. Interleukin-3 (IL-3)

- It is a growth factor for bone marrow stem cells produced by T-cells
- It stimulates multilineage haematopoiesis, and therefore it is also known as the multicolony stimulating factor (multi-CSF)

D. Interleukin-4 (IL-4)

- It is produced by T-helper (Th) cells
- It acts as B-cell differentiating factor, also acts as a growth factor for T-cells and mast cells and enhances the activity of cytotoxic T-cells
- It increases synthesis of IgG-1 and IgE and may play a role in atopic hypersensitivity

E. Interleukin-5 (IL-5)

- It is produced by T-helper (Th) cells
- It causes proliferation of activated B-cells and eosinophils and stimulates production of IgA and IgM

F. Interleukin-6 (IL-6)

- It is produced by stimulated T and B-cells, macrophages and fibroblasts
- It promotes differentiation of B-cells into Ab-producing plasma cells and encourages IgG production
- It acts as an inflammatory response mediator in host defense against infections

G. Interleukin-7 (IL-7)

- It is produced by the spleen, bone marrow stromal cells (BMSCs)
- It is B and T-cells growth factor

H. Interleukin-8 (IL-8)

- It is produced by macrophages and other cells
- It acts as neutrophil chemotactic factor

I. Interleukin-9 (IL-9)

- It is produced by T-cells
- It helps in cell growth and proliferation

J. Interleukin-10 (IL-10)

- It is produced by T and B-cells and macrophages
- It inhibits interferon production and functions of mononuclear cells

L. Interleukin-11 (IL-11)

- It is produced by bone marrow stromal cells
- It induces acute phase proteins

M. Interleukin-12 (IL-12)

- It is produced by T-cells
- It activates NK cells

N. Interleukin-13 (IL-13)

- It is produced by T-cells
- It inhibits functions of mononuclear cells

O. Mitogenic or blastogenic factor (MF or BF)

- It is released by sensitized T-cells stimulated by specific antigen
- It induces nonspecific blast transformation of normal unsensitized T lymphocytes

P. Transfer factor (TF)

- It is an extract from specific antigen-sensitized lymphocytes that mediates passive transfer of CMI is known as transfer factor
- It is a low molecular weight (2000–4000) substance, which is stable at 37°C, –20°C and in the lyophilized form at 4°C, but inactivated at 56°C in 30 minutes
- It is resistant to treatment with DNAase, RNAase and trypsin and freeze thawing
- Chemically, it is a polypeptide-polynucleotide
- It is immunologically specific
- It is highly potent, an extract from 0.1 ml of packed leucocytes is sufficient to transfer CMI
- It is useful in immunocompromised individuals to restore specific CMI, e.g. in
 - T-cell deficiency (Wiskott–Aldrich syndrome, Nezelof syndrome, DiGeorge syndrome)
 - Disseminated infections associated with deficient CMI (tuberculosis, lepromatous leprosy, mucocutaneous candidiasis, etc.)
 - Cancer—melanoma, sarcoma, etc.

2. Cytokines Affecting Macrophages

Antigenically stimulated T - lymphocytes produce certain biologically active soluble proteins (lymphokines), which recruit, activate and regulate effector cells with the potential to fight with the infecting agent.

The biological activities of lymphokines are as follows:

A. Macrophage chemotactic factor (MCF)

It is chemotactic for mononuclear phagocytes and causes accumulation of these cells at the site of Ag-mediated lymphokines' release

B. Migration-inhibiting factor (MIF)

It inhibits migration of phagocytic cells and localizes circulating and tissue monocytes at the site of infection

C. Interferon- γ (IFN- γ)

- It is also known as macrophage-activating factor (MAF)
- It possesses powerful macrophage activating molecules, produces significant morphological changes and also increases content of lysosomal enzymes and their activities so that the macrophage kills ingested intracellular foreign particles

D. Interferon- α (IFN- α)

It is produced by leucocytes and has antiviral activity

E. Interferon- β (INF- β)

It is produced by fibroblasts and inhibits replication of virus in the host cell

3. Cytotoxic Cytokines**A. Lymphotoxin (LT)**

- It is a cytotoxic protein released by activated Th cells
- It is also known as tumour necrosis factor- β (TNF- β)
- It is cytotoxic for foreign cells such as tumour cells, transplanted cells and microorganisms

B. Tumour necrosis factor- α (TNF- α)

- It is produced by macrophages and monocytes. It causes lysis of tumour cells
- It also plays a role in elimination of certain bacteria and parasites

4. Other Cytokines**A. Skin reactive factor (SRF)**

- It is produced specifically by Ag sensitized lymphocytes
- When injected into the skin of normal guinea pig, it produces an indurated and erythematous lesion within 3 hours
- It facilitates the movement of monocytes from blood vessels into extravascular spaces and induces skin hypersensitivity

B. Lymphocyte inhibitory factor

It has T-suppressor activity, suppresses Ab production

■ What are cytotoxic T - cells?

- A population of killer (K) T - cells capable of killing or lysing target cells to which they bind are known as cytotoxic T - cells
- They are formed in response to viral infection and graft from genetically dissimilar member
- They are cytotoxic for host cells infected with virus, graft tissue and tumour cells

■ Mention the tests used for detection of CMI.

- For detection of CMI, skin test was the only method available till recently
- Several tests are now available for detection of CMI. These include:
 - **Lymphocyte transformation test** in which there is a transformation of cultured sensitized T lymphocytes on contact with specific Ag to blast cells—evidenced by enhanced DNA synthesis
 - **Target cell destruction** in which there is a killing of cultured cells by lymphocytes sensitized against them
 - **Migration inhibiting factor test**—it is the most commonly used test for detection of CMI. In this test, macrophages packed in a capillary tube when placed in a tissue culture medium in a chamber, migrate out, and spread over the glass walls of the chamber and form a lacy fan-like appearance. If macrophages are from sensitized guinea pig, the addition of Ag to the culture chamber will inhibit the migration. The test has been adopted for clinical use by incubating human peripheral leucocytes in capillary tubes to culture chambers. When specific antigen is added, the migration of leucocytes is prevented. By comparing with the control test, a semi-quantitative assessment of migration inhibition is possible
 - **Other tests** include: Detection of T cells by SRBC rosette test and by immunofluorescence test

■ Mention the role of CMI.

CMI plays an important role in

- Immunity against infectious diseases caused by obligate and facultative intracellular pathogens
- Delayed hypersensitivity
- Transplantation immunity and graft versus host reaction
- Immunological surveillance and immunity against cancer
- Pathogenesis of certain autoimmune diseases such as thyroiditis, encephalomyelitis, etc.

■ What is immunological tolerance?

Tolerance is a specific immunological unresponsiveness. The capacity of an animal to respond to a particular Ag can be inhibited while allowing it to respond to other Ags. This is possible if the animal is exposed to the Ag in early life or exposed to a very large dose of Ag in latter life. This induced inability of the animal to respond to Ag, to which it would be expected to respond normally, is known as immunological tolerance. Thus, it is a state of unresponsiveness, specific for a particular Ag, induced by prior exposure to that Ag.

■ Mention different types of immunological tolerance and factors responsible for it.

- Tolerance may be total or partial, short-lived or long-lasting
- The induction, degree and duration of tolerance depend on
 - **Species of animals**—rabbits and mice are more readily tolerated than guinea pigs
 - **Immunocompetence of the host**
 - **Nature of the Ag**—soluble Ags and haptens are more readily tolerated than particulate Ags
 - **Dose of Ag**—larger dose of Ag is required, also further increase in dose increases the duration of tolerance
 - **Route of administration**—the best route is that which equilibrates Ag throughout the extra and intravascular compartments. For Ags which do not equilibrate readily or are rapidly eliminated, the best route is intravenous route
- Two types of doses can induce tolerance—high dose of Ag produces high zone tolerance and low dose of Ag produces low zone tolerance
- Intermediate dose induces immunity
- A special type of high zone tolerance is immunological paralysis
- The duration of tolerance is variable. Tolerance can be prolonged by repeated tolerogenic stimuli

■ Discuss the mechanism of immunological tolerance.

- In both, neonates and adults, the mechanism of tolerance induced is same
- In both, the tolerance-inducing dose of Ag is significantly more than the dose of Ag required for immunization
- A greater difficulty in inducing the tolerance in the adult may arise due to large number of immunocompetent cells
- The exact mechanism is not clearly known. The possible mechanisms, which may act singly or in combination are as follows:

1. Clonal Deletion

Burnet, on the basis of clonal selection model, postulated that during the developmental phase, the lymphocyte goes through a phase in which contact with Ag results into death or a permanent

inactivation of that lymphocyte instead of its stimulation, which leads to immunological tolerance.

2. T-suppression

Tolerance can be induced by T-suppressor cells directed against T-helper cells. T-suppressor cells may act on T-helper cells and inhibit lymphocyte clone reactivity, thereby inducing tolerance.

3. Helplessness

T-cells are more readily tolerated than B-cells. T-cells are necessary to help B-cells to produce Abs by T-dependent Ags. These Ags require help of T-cells to provide proper signal to B-cells. When T-cells are tolerated then B-cells become helpless to induce Ab formation. The result is failure to produce Ab and this results in induction of tolerance.

4. Genetic Unresponsiveness

If an individual lacks genetic programme required for development of immune response, he will remain immunologically silent.

No genes → No recognition → No immune response → Tolerance

5. Other Ways of Inducing Tolerance

The alternative explanations are:

- Interaction of immunocompetent cells, with the excess of an Ag so that no information is given to Ab-producing cell
- An Ag reacts with the Ab-producing cells but blocks the transmission of an informational molecule
- When the Abs synthesized are neutralized or destroyed
- Inability of macrophages to present the Ag to T-cells effectively
- Development of tolerance involves both, antibody-mediated and cell-mediated immunity simultaneously. When tolerance is induced to either AMI or CMI without affecting the other then it is called **split tolerance**. In guinea pigs, injection of tuberculin prior to BCG vaccination can inhibit delayed hypersensitivity to tuberculin without affecting the production of circulating Ab. Split tolerance can be induced by special techniques

Artificially Induced Tolerance

Tolerance can be induced artificially by

- Administration of antisera or antibody
- Cytotoxic drugs
- Surgical ablation

23

Chapter

Immunodeficiency Diseases

■ Define and classify immunodeficiency diseases.

- Immunodeficiency disorders are conditions where defense mechanisms of the host are impaired leading to increased susceptibility to microbial infections and sometimes enhanced susceptibility to malignancies and autoimmune diseases
- Deficiencies of immune system may involve:
 1. Specific immune functions—humoral immunity (HI), cell-mediated immunity (CMI) or both
 2. Nonspecific immune functions—phagocytosis and complement

Immunodeficiencies may be classified as:

Primary Immunodeficiencies

These disorders are due to abnormalities in the development of immune mechanisms. These are genetically determined and may be congenital.

Secondary Immunodeficiencies

These disorders are due to diseases, drugs, nutritional inadequacies and other processes that interfere with proper functioning of the mature immune system.

■ Give a detailed description of primary immunodeficiencies.

Primary immunodeficiencies involve:

1. Deficiency of innate immunity
2. Humoral immunodeficiencies
3. Cellular immunodeficiencies
4. Combined immunodeficiencies

Deficiency of Innate Immunity

Phagocytes and complement are the major components of innate immunity. They may be impaired leading to various disorders.

Disorders of Phagocytosis

The defects in the phagocytic function may be due to

1. Extrinsic factors—such as opsonic antibody, complement or other factors promoting phagocytosis
2. Intrinsic factors—such as enzyme deficiencies

Examples

Chronic granulomatous disease

- The monocytes and polymorphonuclear leucocytes fail to produce hydrogen peroxide due to defect in NADPH oxidase because of which they fail to kill ingested organisms intracellularly

- Result: Susceptibility to recurrent infections by catalase positive pathogens such as staphylococci and coliforms increases

Chediak-Higashi disease

The lysosomes are structurally and functionally abnormal; hence, do not interact with microbes. This results in pyogenic infections, which can be fatal.

Myeloperoxidase deficiency

Leucocytes have reduced myeloperoxidase activity that results in increased susceptibility to candidiasis.

Lazy leucocyte syndrome

Neutrophils are unable to respond to chemotactic stimuli (become lazy), hence increased susceptibility to bacterial infection with recurrent stomatitis, gingivitis and otitis.

Tuftsinn deficiency

Tuftsinn is a leucokinin-stimulating phagocytosis. Its deficiency increases susceptibility to local and systemic bacterial infections.

Leucocyte glucose 6-phosphate dehydrogenase (G6PD) deficiency

Leucocytes are deficient in G6PD. Hence, bactericidal activity after phagocytosis is diminished and leucocytes are unable to destroy engulfed bacteria that increase susceptibility to microbial infections.

Other disorders

Other disorders of the phagocytosis include:

- Shwachman's disease
- Hyper IgE syndrome
- Actin-binding protein deficiency
- Job's syndrome

Disorders of Complement Components

Complement components are necessary for neutrophil chemotaxis, opsonization and bacterial killing. Complete or partial deficiency of C components, except C9 and complement inhibitors, may occur. Genetic deficiencies of complement components increase susceptibility to microbial infections and autoimmune diseases.

Examples

- Deficiency of C1q—SLE (systemic lupus erythematosus)-like syndrome
- Deficiency of C1r—glomerulonephritis, polyarthritis and SLE-like syndrome
- Deficiency of C1s, C4 and C2—SLE-like syndrome
- Deficiency of C3 and its regulatory protein C3b inactivator—severe recurrent pyogenic infections by bacteria
- Deficiency of C5 to C8—bacteraemia, mainly with Gram-negative diplococci (Neisserial infections) and toxoplasmosis
- Deficiency of factor H and factor I—Susceptibility to infection
- Deficiency of factor D and properdin—Neisserial infections

Deficiency of complement inhibitors

- Deficiency of C1 inhibitor—hereditary angioneurotic oedema. It is inherited as an autosomal dominant defect
- Deficiency of C3b inactivator—chronic recurrent pyogenic infections

Humoral Immunodeficiencies

- These are defects in antibody-producing B-cells that lead to deficiencies of B-cells due to nonproduction of B-cells or due to interference with the maturation process of B-cells
- Result is gross depletion of all immunoglobulins in the serum and a marked decrease in the number of B-cells in circulation that leads to increased susceptibility to bacterial infections such as staphylococcal, streptococcal, pneumococcal, meningococcal, pseudomonal and *H. influenzae* infections
- Infection by *Pneumocystis carinii* and autoimmune diseases are also seen
- CMI remains normal and viral infections, e.g. measles are readily brought under control

Disorders of Humoral Immunodeficiency***X-linked agammaglobulinaemia (Bruton's diseases)***

In this disorder B-cells fail to develop into mature B-cells. This results in absence of B-cells and depletion of all classes of immunoglobulins that result in increased susceptibility to severe recurrent infections by pyogenic bacteria.

Transient hypogammaglobulinaemia of infancy

There is an abnormal delay in the synthesis of IgG, which is normally initiated by the second month. This delay increases susceptibility of the infant to recurrent otitis media and respiratory tract infections.

Selective immunoglobulin deficiency (dysgammaglobulinaemia)

- There is selective deficiency of one or the other immunoglobulin classes. The isolated IgA deficiency is most common (0.2% in normal population) and associated with increased susceptibility to respiratory infection and chronic diarrhoea
- The selective IgM deficiency is associated with septicaemia and IgG deficiency is associated with respiratory diseases

Immunodeficiencies with hyper-IgM

- There is elevated IgM level while IgA and IgG levels are low
- Result: Susceptibility to infections and autoimmune disorders

Transcobalamin II deficiency

- Transcobalamin II is a serum protein involved in the transport of vitamin B₁₂
- Deficiency associated with metabolic effects of vitamin B₁₂ deficiency and is characterized by diarrhoea, anaemia, agammaglobulinaemia, etc.

Cellular Immunodeficiencies

- Deficiency of cell-mediated immune response
- The thymus-dependent areas of lymph nodes and spleen are depleted of T-cells, and circulating T-cells are also reduced in number
- Delayed hypersensitivity and graft rejection are depressed while humoral immunity may or may not be affected

- Individuals become susceptible to infections by viruses, intracellular bacteria, fungi and protozoan parasites

Disorders

DiGeorge syndrome

This occurs due to thymic aplasia or hypoplasia characterized by

- Neonatal hypocalcaemia
- Severe cardiac disease in early weeks of life
- Fallot's tetralogy
- Facial and midline structure defects
- Affected individuals are susceptible to oral candidiasis, chronic diarrhoea, pneumonia and cutaneous infections

Chronic mucocutaneous candidiasis

It occurs due to selective cellular immunodeficiency to *Candida albicans*, characterized by severe chronic candidiasis of the mucous membranes, skin and nails

Purine nucleoside phosphorylase deficiency (PNP)

- The enzyme PNP is involved in the metabolism of purines
- Its deficiency is associated with decreased CMI
- Results in recurrent or chronic infections such as pneumonia, diarrhoea and candidiasis

Combined Immunodeficiencies (Stem Cell Deficiency)

- These are characterized by abnormal functioning of B and T-cells
- There is marked deficiency of T-cells and varying degrees of deficiency of B-cells
- Patients are susceptible to recurrent fungal, bacterial, viral, protozoal infections and autoimmune diseases

Disorders

Nezelof syndrome (cellular immunodeficiency with abnormal immunoglobulins)

- CMI is depressed and immunoglobulin levels are selectively elevated, decreased or normal
- Patients are susceptible to pulmonary infections, oral or cutaneous candidiasis, chronic diarrhoea, recurrent microbial infections and autoimmune haemolytic anaemia

Ataxia telangiectasia

- This is an autosomal recessive syndrome characterized by cerebellar ataxia, ocular and cutaneous telangiectasia and sinopulmonary infection in early life, endocrine abnormalities and predisposition to malignancy in the second or third decade
- In most patients IgA is deficient. IgE deficiency is also frequent and CMI is also defective

Wiskott-Aldrich syndrome

- It is characterized by thrombocytopenic purpura, eczema, recurrent infections by various microorganisms including herpes simplex, Cytomegalovirus, *Pneumocystis carinii* and varicella virus infections
- Death occurs due to infection, haemorrhage or lymphoreticular malignancy
- There is a progressive deterioration of CMI. Serum IgM level is low but IgG and IgA levels are normal or elevated and IgE is markedly elevated

Good's syndrome (immunodeficiency with thymoma)

- This occurs in adults after 40 years of age and is associated with a benign or malignant thymic tumour, impaired CMI and agammaglobulinaemia
- Patients suffer from sinopulmonary infections, septicaemia, urinary tract infections, chronic diarrhoea, dermatitis and aplastic anaemia

Severe combined immunodeficiencies

- Include many syndromes with severe deficiency of both HI and CMI
- Many distinct patterns have been described, these are: the Swiss type agammaglobulinaemia, reticular dysgenesis of de Vaal and adenosine deaminase deficiencies

Immunodeficiency with short-limbed dwarfism

- In this disorder CMI is defective due to thymic defects resulting from ectodermal dysplasia
- Patients show increased susceptibility to infection

Episodic lymphopenia with lymphocytotoxin

- In this disorder, there is depression of T-cells function (episodic) by the action of a circulating complement-dependent lymphocytotoxin (antilymphocyte antibody)
- The patients lack immunological memory, so that the secondary antibody response is abolished

- **List the diagnostic tests employed for determining (a) disorders of phagocytosis, (b) B-cell deficiency, (c) T-cell deficiency and (d) combined immunodeficiency. Also give the line of treatment followed for the aforementioned diseases.**

Diagnosis***(a) Disorders of phagocytosis***

- Examination of blood—differential count
- Examination of bone marrow for abnormalities like number of phagocytic cells (count) and abnormal morphological changes
- Analysis of phagocytic cell activity by nitroblue tetrazolium dye reduction test

(b) B-cell deficiency

Quantitation of serum immunoglobulin levels.

(c) T-cell deficiency

- Skin test—patients with T-cells immunodeficiency will be hypo or unreactive in skin test to microbial antigens
- Enumeration of T-cells by SRBC rosette test

(d) Combined immunodeficiency

Quantitation of immunoglobulin levels and enumeration of T-cells.

Treatment***(a) Deficiency of innate immunity***

Antibiotics to treat pyogenic infections.

(b) B-cell deficiency

Immune serum globulin.

(c) T-cell deficiency

Thymus transplantation, transfer factor therapy or injections of thymosin.

(d) Combined immunodeficiencies

Bone marrow transplantation, transfer factor therapy and thymus transplantation.

■ **Write a short note on secondary immunodeficiencies.**

- Immune responsiveness can be decreased nonspecifically by many factors such as malnutrition, malignancies, microbial infections, chronic diseases, metabolic disorders, immunosuppressive drug therapy and X-ray irradiation and other factors leading to immunodeficiency. Secondary immunodeficiencies are more common than primary immunodeficiencies
- Secondary immunodeficiency disorders occur due to impairment of **cell-mediated immunity (CMI)** or **humoral immunity (HI)**

Impairment of CMI

CMI may be impaired in a state of malnutrition (iron deficiency in particular), malignancy (Hodgkin's disease), metabolic disorders such as diabetes mellitus, cytotoxic drugs, obstruction to lymph circulation, lymphorrhoea, microbial infections such as lepromatous leprosy, HIV infection, infectious mononucleosis and measles and immunosuppressive therapy.

Impairment of HI

HI may be impaired in lymphoid malignancy (chronic lymphatic leukaemia), nephrotic syndrome (immunoglobulin catabolism), exfoliative skin disease (loss of serum protein), multiple myeloma, immunosuppressive therapy (corticosteroids and others), malnutrition and exposure to X-rays, etc.

The patients with impaired CMI and HI show increased susceptibility to pyogenic bacteria, tubercle bacillus, brucella, *Cryptococcus*, herpes zoster virus, cytomegalovirus, *Pneumocystis carinii* and other opportunistic pathogens.

■ **Write a short note on acquired immunodeficiency syndrome (AIDS).**

- AIDS is an acquired immunodeficiency leading to a syndrome, which increases susceptibility of affected individuals to various microbial infections and leads to Kaposi's sarcoma—a dermal malignancy
- It is caused by human immunodeficiency virus (HIV)
- The HIV attacks T-helper cells (CD-4 lymphocytes) which leads to reduction in number of these cells, thus causing loss of immunological protection
- Infected individuals show increased susceptibility to overwhelming opportunistic infections and develop Kaposi's sarcoma and other malignancies, autoimmune diseases, intestinal infections, neurological problems, lymphadenopathy and many other symptoms

24

Chapter

Hypersensitivity (Allergy)

■ Explain hypersensitivity.

Immune response is generally beneficial to the host but sometimes it may be harmful. Sensitized individuals may respond to subsequent antigenic stimuli in heightened or exaggerated manner that is harmful to the host and lead to tissue damage, disease and even death of the individual. This type of response is termed hypersensitivity or allergy. The term allergy (meaning altered response) was coined by Clemens von Pirquet (1906) to cover any altered response to an antigen.

Increased resistance is called immunity and increased susceptibility is called hypersensitivity.

In allergic (hypersensitivity) reactions:

- An antigen—is referred to as allergen or sensitizer
- Immunization—is referred to as sensitization
- An individual—is referred to as hypersensitive or allergic

■ Classify the hypersensitivity reactions based on the time taken by them to develop.

Based on the time required for a sensitized host to develop clinical reactions upon re-exposure to the Ag, hypersensitivity reactions are classified into two types:

1. Immediate hypersensitivity
2. Delayed hypersensitivity

Immediate Hypersensitivity

It is a hypersensitive state in which an allergic reaction develops immediately after contact with an Ag (generally in few minutes to few hours, sometimes within a few seconds). It is a B cell- or Ab-mediated reaction, e.g. anaphylaxis, Ab-mediated cytotoxic hypersensitivity and Ag-Ab complex-mediated hypersensitivity.

Delayed Hypersensitivity

It is a hypersensitive state in which there is an appreciable delay (about 24–72 hours) between the exposure to the Ag and development of symptoms. It is a T-cell-mediated reaction, e.g. infection (tuberculin) type and contact dermatitis type of reactions.

■ Differentiate between immediate type and delayed type of hypersensitivity reactions.

Features distinguishing immediate type hypersensitivity from delayed type are presented in Table 24.1.

Table 24.1 Differences between immediate and delayed types of hypersensitivity

Immediate	Delayed
1. Appears immediately within short time and recedes rapidly	Appears slowly and lasts longer
2. Induced by Ag or hapten by any route	Induced by Ag or hapten intradermally or with Freund's adjuvant or by skin contact
3. Antibody mediated reaction	Cell mediated reaction
4. Passive transfer with serum	Passive transfer with lymphocyte or transfer factor
5. Desensitization easy but short-lived	Desensitization difficult but long-lasting

■ What is the basis of Coombs and Gel classification of hypersensitivity reactions?

Coombs and Gel (1963) classified hypersensitivity reactions on the basis of mechanisms of pathogenesis.

According to their system of classification hypersensitivity reactions are of four types. These are, as follows:

1. Type I—Anaphylaxis (IgE or reagin-dependent)
 2. Type II—Ab-mediated cytotoxic hypersensitivity
 3. Type III—Immune complex-mediated hypersensitivity
 4. Type IV—Delayed or cell-mediated hypersensitivity
- One additional type has been recently proposed
5. Type V—Stimulatory hypersensitivity

■ Discuss in detail Type I hypersensitivity reaction (anaphylaxis).

Type I/Anaphylaxis (IgE or reagin-dependent)

It is an IgE-mediated immediate type of hypersensitivity reaction. The term anaphylaxis (*ana* means without, *phylaxis* means protection) was coined by Richet (1902) to describe his experiment on dog. Smith (1902) had noticed a similar phenomenon in guinea pigs, following injections of toxin-antitoxin mixtures.

- In this reaction, when a guinea pig is injected with an antigen (about 1 mg) such as egg albumin, no adverse effects are noted but when a second dose of same antigen (egg albumin) is injected intravenously after an interval of 2–3 weeks, the sensitized guinea pig reacts dramatically and the condition known as '*anaphylactic shock*' is developed
- The animal becomes restless, cyanosed, may develop convulsions and die
- The initial injection of antigen is termed *sensitizing dose*. It is more effective by parenteral route than other routes
- The second injection of antigen is termed *shocking dose*. It is more effective by intravenous route than other routes
- During an interval between the two injections, the animal forms antibodies
- Anaphylaxis is the result of interaction of the shocking dose of antigen with newly formed antibodies on the surface of tissue cells
- This interaction triggers the release of pharmacologically active substances, which increase capillary permeability and cause smooth muscle contraction

Mechanism of Anaphylaxis

- The cell types of greatest importance are the mast cells normally present in submucosal layers of respiratory tract, gastrointestinal tract, skin and vascular endothelium. Basophils of blood also participate in the reaction
- Reaginic antibodies belonging to the class IgE possessing specific configuration and having a strong affinity for tissue cells are responsible for anaphylaxis.
- The first step is the synthesis of antibodies capable of binding to mast cells and basophils. For this, an antibody must possess a specific configuration, which is complementary fit for specific receptor site on mast cells and basophils. The IgE antibodies coat the mast cells and basophils with the help of Fc region and bind specifically to sites on the mast cell surface
- The second step in anaphylaxis is the combination of the cell-fixed antibodies (Fab fractions) with specific antigen of shocking dose, bridging the gap between adjacent antibody molecules on cell. This interaction increases the permeability of the cells to calcium ions and causes degranulation through proteolytic enzymes, which leads to the release of pharmacologically active substances present in the granules (Fig. 24.1).
- The mechanism of anaphylaxis is summarized in Flowchart 24.1.

■ Which are the two types of pharmacological mediators of anaphylaxis?

Pharmacological mediators are of two types:

1. **Primary mediators**—are the preformed contents of mast cell and basophilic granules. These include histamine, serotonin, and eosinophil chemotactic factor of anaphylaxis, neutrophils chemotactic factor of anaphylaxis, heparin and various proteolytic enzymes
2. **Secondary mediators**—are newly formed upon stimulation by mast cells, basophils and other leucocytes. These include slow reactive substance of anaphylaxis, prostaglandins and platelet activating factors

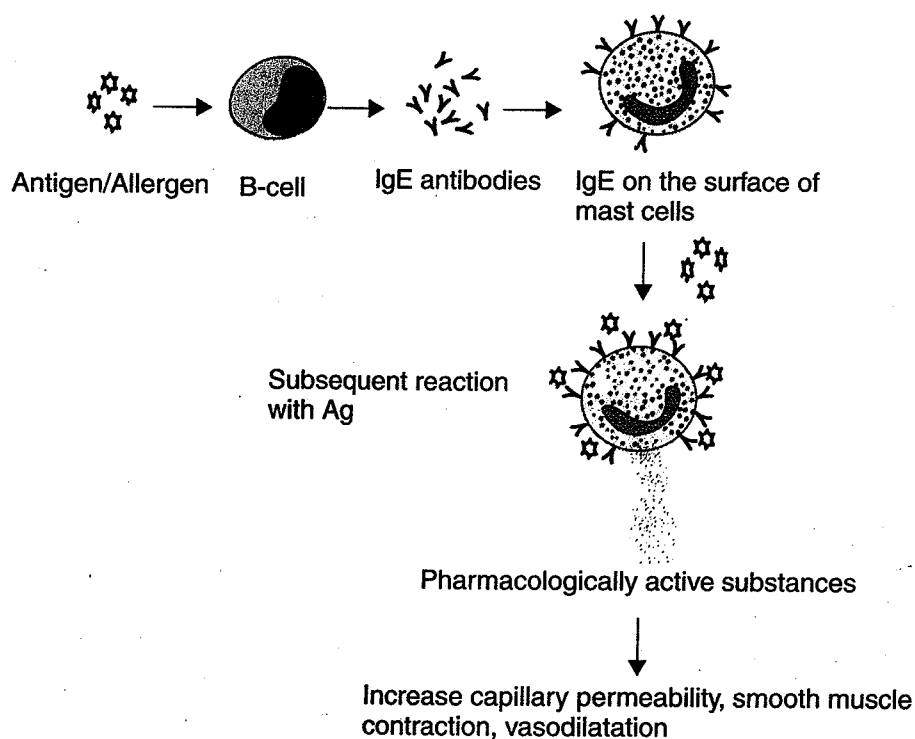


Fig. 24.1 Mechanism of Anaphylaxis

First step

Synthesis of IgE antibodies possessing a specific configuration complementary fit for specific receptor site on mast cells and basophils

The IgE antibodies coat the mast cells and basophils with the help of Fc region and bind specifically to sites on the mast cell surface

Second step

Combination of the cell-fixed antibodies (Fab fractions) with specific antigen of shocking dose

Bridging the gap between adjacent antibody molecules on cell

This interaction increases the permeability of the cells to calcium ions and causes degranulation through proteolytic enzymes

Leads to the release of pharmacologically active substances present in the granules

Primary mediators

Histamine, serotonin, eosinophil chemotactic factor of anaphylaxis, neutrophils chemotactic factor of anaphylaxis, heparin and various proteolytic enzymes

Secondary mediators

Slow reactive substance of anaphylaxis, prostaglandins, platelet-activating factors

Flowchart 24.1 Mechanism of anaphylaxis.

- Write short notes on the following mediators of anaphylaxis: (a) Primary mediators, (b) Secondary mediators and (c) Mediators other than these, if any.

Primary Mediators**1. Histamine**

It is formed by decarboxylation of histidine present in the granules of mast cells, basophils and platelets. It induces smooth muscle contraction, and increases capillary permeability and vasodilatation

2. Serotonin (5-hydroxy tryptamine)

- It causes smooth muscle contraction, increases capillary permeability and also causes vasoconstriction in animals
- It does not play any significant role in human beings

3. Eosinophil chemotactic factors of anaphylaxis (ECFA)

- Released by mast cells and basophils
- Chemotactic for eosinophils—probably contribute to the eosinophilia
- Also enhance C3b activity and cause antibody and complement-dependent damage to some parasites

Secondary Mediators**1. Slow reactive substance of anaphylaxis (SRS-A)**

- An acidic lipid (leukotrienes) more potent bronchoconstrictor than histamine
- Its action is slow, prolonged and is not inhibited by antihistamines. It is a predominant pharmacological mediator in human asthma

2. Prostaglandins and thromboxanes

- Derived from mast cells and other leucocytes
- Prostaglandin E₂ is a bronchodilator
- Prostaglandin F_{2a} and thromboxane are transient bronchoconstrictors

3. Platelet activating factor (PAF)

- Lipid released by basophils and mast cells. It causes aggregation of platelets and release of their vasoactive amines

Other Mediators of Anaphylaxis Include Kinins

1. Polypeptides produced by the action of proteolytic enzymes on kininogen
2. Cause smooth muscle contraction, increased vascular permeability, vasodilatation and pain
3. The best-known kinin is bradykinin. Its role in human anaphylaxis is not known

■ What is systemic anaphylaxis?

- Systemic anaphylaxis is a condition of an **acute shock usually terminating in death** following the injection of an antigen into a previously sensitized animal. Death occurs due to suffocation from contraction of muscles in the walls of bronchioles
- Injecting serum from sensitized animal can passively transfer this reaction and then the animal can be shocked by antigen, which is used for sensitization (passive systemic anaphylaxis)

Systemic Anaphylaxis in Human Beings

- Systemic anaphylaxis in man occurs following insect bite such as bee or wasp stings, injection of antitoxic serum such as anti-tetanus (ATS), anti-diphtheritic (ADS) or anti-gas gangrene serum (AGS) and penicillin
- In humans, reaction is characterized by severe respiratory distress due to bronchiolar constriction with vascular collapse, laryngeal oedema, acute hypotension, loss of consciousness and death

■ Discuss cutaneous anaphylaxis (local anaphylaxis). Briefly discuss the mechanism of development and desensitization process of this reaction.

Cutaneous or Local Anaphylaxis

- A local wheal and flare reaction may be induced by intradermal injection of antigen into actively sensitized host. This is known as cutaneous or local anaphylaxis
- The wheal is a pale, central area of puffiness due to oedema, which is surrounded by a flare caused by hyperaemia and subsequent erythema
- This test can be used as skin test for testing Type I hypersensitivity and to identify the allergen responsible for atopic diseases
- In highly sensitized individuals, the skin test may lead to systemic anaphylaxis terminating in death; hence a syringe loaded with adrenalin should be kept ready before the test is performed

Passive Cutaneous Anaphylaxis

The cutaneous anaphylaxis may also be induced passively by the intradermal injection of antibodies. Local wheal and flare type reaction may then be demonstrated by intravenous injection of antigen with dye such as Evan's blue after 4–24 hours. The combination of antigen and antibody increases capillary permeability and vasodilatation permitting the escape of Evan's blue at the site of intradermal injection resulting in immediate bluing. This is passive cutaneous anaphylaxis and can be used as an extremely sensitive *in vivo* method for detection of antibodies.

Local Anaphylaxis in Human Beings (Atopy)

- Hypersensitive state in which a person reacts with substances encountered during the course of everyday life is known as atopy (meaning out of place or strangeness) or idiosyncrasies. The term atopy was coined by Coca (1923)
- About 10% of the population suffers from atopy to allergens such as grass pollen, animal danders, mites in house, dust, food, etc.
- Contact of allergen with cell-bound IgE in the bronchial tree, the nasal mucosa, the conjunctival tissue, intestine or skin, releases pharmacologically active mediators and produces symptoms of
 - Asthma
 - Hay fever (allergic rhinitis)
 - Conjunctivitis
 - Gastrointestinal symptoms
 - Dermatitis
 - Urticaria in persons allergic to food such as strawberry

Mechanism

- Atopic sensitivity is due to **excessive production of IgE antibodies** and often associated with deficiency of IgA
- The lymphocytes responsible for synthesis of IgA and IgE are parallelly distributed in the respiratory and intestinal submucosa
- In normal individuals, the antigen interacts with IgA-producing lymphocytes and hence the IgE-producing lymphocytes do not come in contact of antigen
- When IgA is deficient, an Ag causes massive stimulation of IgE-producing lymphocytes, leading to overproduction of reagins responsible for the clinical expression of atopic reactions

Desensitization

Desensitization in atopy can be achieved by injecting serum (acute or temporary desensitization) or by giving repeated injections of antigen (prolonged desensitization). Alternatively, allergen with oil adjuvant (depot therapy) can be used for prolonged desensitization.

■ Write a short note on anaphylaxis in vitro.

Isolated tissues such as uterus or ileum from sensitized guinea pigs, held in a bath of ringer solution or isotonic fluid will contract vigorously on addition of the specific antigen. This is known as **Schultz–Dale phenomenon**. Tissues from normal animals can be passively sensitized by treatment with serum from sensitized animal.

■ What is anaphylactoid reaction?

- It is a type of reaction that clinically resembles anaphylactic shock
- It develops following—the intravenous injection of peptone, trypsin, heavy metal salts, starch, or polysaccharide
- Its clinical resemblance is due to the same chemical mediators participating in both the reactions
- This reaction has no immunological basis and occurs nonspecifically due to the activation of the alternate complement pathway and release of anaphylatoxin

■ Write a short note on Type II hypersensitivity reaction.

Type II/Cytotoxic Reaction

- In this type of reaction, antigenic component is either a part of tissue cell, or a microbial product or a drug attached to cell wall

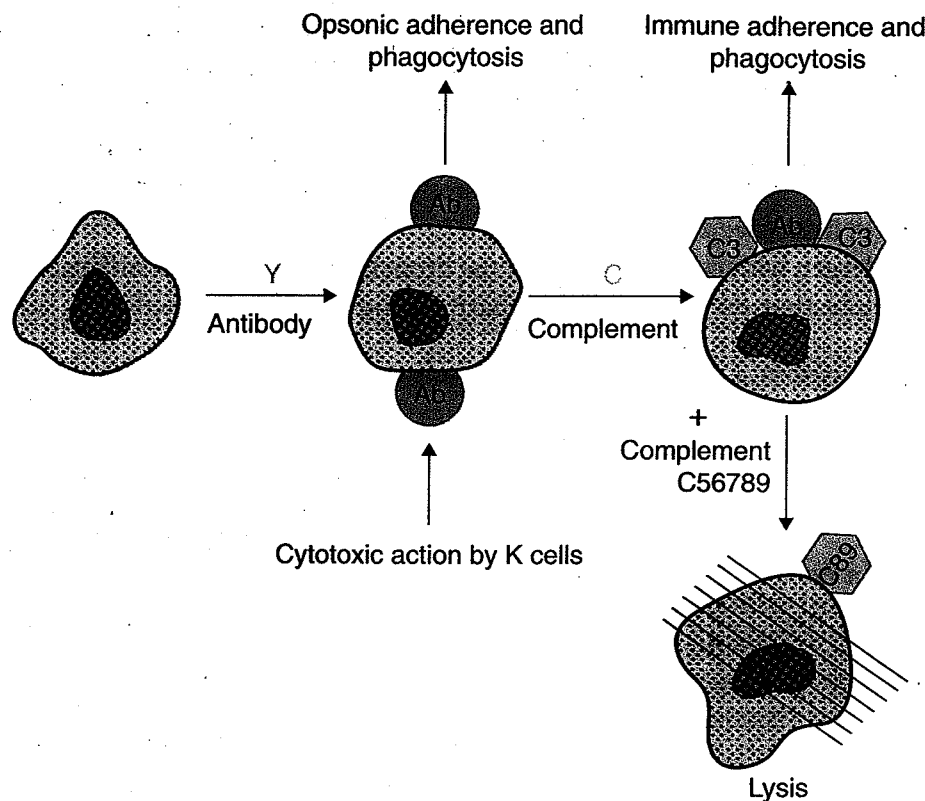


Fig. 24.2 Cytotoxic hypersensitivity.

- Combination of this antigenic component with IgG or IgM antibody produces damage to cell by promoting contact with phagocytic cell or by activating complement components
- Contact with phagocytic cells is promoted by reduction in surface charges, by opsonic adherence directly through Fc or by immune adherence through bound C3
- Cell death may also occur through activation of full complement pathway up to C8 and C9 (Fig. 24.2)

Examples

1. Lysis of red blood cells by antierythrocyte antibodies
2. Lysis of platelets by autoantibodies leading to autoimmune thrombocytopenia
3. Drugs such as penicillin, sulphonamides, quinidine, sedormid, etc. attach to the surface of cells such as RBCs, neutrophils or platelets. This causes change in antigenicity, inducing antibody synthesis, which causes cytotoxic reaction. Classical example is **thrombocytopenic purpura** produced by sedormid (sedormid purpura)

■ Describe Type III hypersensitivity reaction.

Type III/Immune Complex-mediated Hypersensitivity

- It is characterized by deposition of antigen-antibody complexes in tissues, particularly on endothelial surfaces
- The complex formation in and around small blood vessels may result in acute inflammatory reactions. Sometimes they cause mechanical blockage of the vessels because of platelet aggregation causing interference with blood supply to surrounding tissues
- This antigen-antibody complex may activate complement-causing release of anaphylatoxin that causes release of histamine responsible for vascular permeability changes
- Activation of complement also causes aggregation of polymorphonuclear leucocytes (PML), which engulf Ag-Ab complexes and release proteolytic enzymes and polycationic proteins. This results in increased capillary permeability

- Activation of complement and massive infiltration by PML and attraction of platelets lead to **inflammation and tissue injury**
- It is of the following two types:
 1. Arthus reaction
 2. Serum sickness

Arthus Reaction (Arthus 1903)

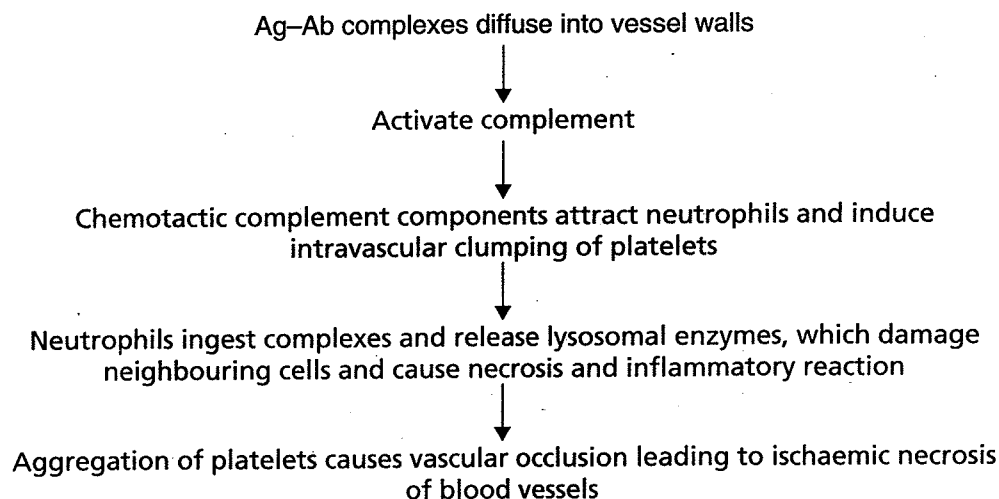
- It is a local inflammatory reaction with particular involvement of blood vessels. It occurs following the injection of Ag (horse serum) intradermally or subcutaneously in hyperimmunized animal (animal which has received several injections of horse serum and developed high levels of Abs-IgG). The reaction presents as an erythema, oedema, induration and haemorrhagic necrosis (see Flowchart 24.2).
- In humans, Arthus-like reaction is produced in individuals who have received several injections of antitoxic sera or insulin.

Serum Sickness (Clemens von Pirquet and Schick 1905)

- It is a systemic reaction that may occur in man following the injection of horse serum and sometimes following the injection of drugs such as sulphonamide, penicillin, streptomycin or organic arsenicals. A single dose is sufficient to produce symptoms. The symptoms appear after 7–14 days. The symptoms are:
 - Fever
 - Lymphadenopathy
 - Splenomegaly
 - Arthritis
 - Glomerulonephritis
 - Endocarditis
 - Vasculitis
 - Urticaria
 - Abdominal pain
 - Nausea and vomiting

Mechanism

- The pathogenic mechanism—is the formation of Ag–Ab complexes, which get deposited on endothelial lining of blood vessels in various parts of the body causing inflammatory infiltration



Flowchart 24.2 Mechanism of inflammation by Arthus reaction.

- This is self-limited disease, with increase in Ab level; Ag-Ab complexes become larger in size and susceptible to phagocytosis and immune elimination
- The damage to tissue produced is same as in Arthus reaction

■ Describe Type IV hypersensitivity reaction.

Type IV/Delayed Hypersensitivity (Fig. 24.3)

- In this reaction there is an appreciable delay between the exposure to Ag and the development of symptoms
- The reaction is mediated by sensitized T-cells and not by Abs. The reaction is demonstrated by a cutaneous reactivity, which becomes visible after 24–48 hours after the introduction of Ag
- The cutaneous reactions are inflammatory and indurated type involving lymphocytes and macrophages and not wheal and flare type as seen in anaphylaxis
- The reaction is induced by sensitized T-cells, which on contact with the specific Ag, release lymphokines. These lymphokines cause biological effects on leucocytes, macrophages and tissue cells
- Passive transfer—to a normal recipient is possible with the help of sensitized T-lymphocytes from a sensitized donor or with the help of transfer factor and not by serum
- It occurs in the following two forms:
 1. Tuberculin (infection) type
 2. Contact dermatitis type

Tuberculin (Infection) Type

It develops as a result of infection with tubercle bacillus and is demonstrated by tuberculin reaction.

- When a small dose of Ag (tuberculin)—1–5 TU (1 TU, equivalent to 0.01 mg OT or 0.00002 mg PPD) is injected intradermally in an individual sensitized to tubercular protein by previous infection or immunization, an erythema and indurated swelling develops gradually. It reaches maximum size after 24–72 hours
- Histologically, the reaction is characterized by infiltration of injection site with mononuclear cells, mainly lymphocytes and 10–20% macrophages. The inflammatory cells (macrophages and lymphocytes) may be seen around blood vessels and nerves
- This type of reaction is also seen in other chronic or subacute infections caused by intracellular pathogen, e.g. leprosy, brucellosis, etc.

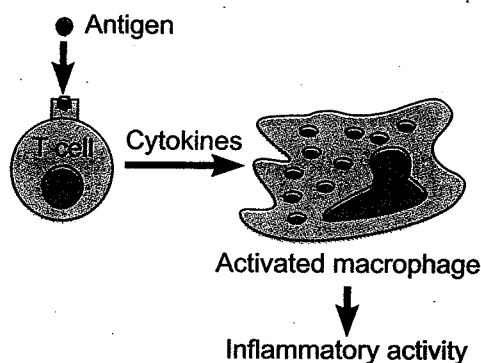


Fig. 24.3 Type IV (delayed/cell-mediated) hypersensitivity.

Contact Dermatitis Type

- It is delayed hypersensitivity reaction that develops as a result of contact of skin with a variety of substances such as
 - Drugs—like penicillin, sulphonamide, organic arsenicals, etc.
 - Metals—like nickel, cobalt, etc.
 - Chemicals—like picryl chloride, dinitrochlorobenzene, formaldehyde, iodine, dyes, soaps, cosmetics, etc.
- These substances are not antigens but act as haptens and combine with skin proteins and become antigenic
- Contact of these substances in sensitized individual leads to contact dermatitis characterized by macules and papules to vesicles that break down leaving behind raw weeping areas typical of **acute eczematous dermatitis**
- Histologically, there is a mononuclear cell infiltration in the upper layers of the skin and around hair follicles
- In this, it may be possible to identify the substance responsible for sensitivity by patch test. In this test the suspected substance is applied to skin under an adherent dressing and observed for itching (after 4–5 hours) and local reaction—erythema to vesicle or blister formation (in 24–28 hours)

■ What is Type V hypersensitivity reaction?

Type V: Stimulatory Hypersensitivity

Certain IgG Abs have ability to stimulate their target cells rather than to kill or inhibit them. These Abs react with the key surface component and stimulate the functional activity of the cell, e.g. thyrotoxicosis (Graves' disease) in which Abs react with thyroid cell and stimulate the activity of these cells. This causes excessive secretion of thyroid hormone that leads to thyrotoxicosis.

■ Briefly describe Schwartzman reaction.

- It is **not an immune reaction** but has superficial resemblance to hypersensitivity
- Schwartzman (1928) observed that injection of a culture filtrate of *S. typhi* (endotoxin) intradermally in a rabbit followed 24 hours later by the same filtrate intravenously results in development of a haemorrhagic necrotic lesion at the site of the intradermal injection
- The initial injection (preparatory dose) causes accumulation of leucocytes, which condition the site by releasing lysosomal **enzymes damaging capillary walls**
- The intravenous dose (provocative dose), which can be of a variety of substances such as bacterial endotoxins, Ag–Ab complexes, starch, serum, kaolin, etc. causes **intravascular clotting, necrosis of vessel walls and haemorrhage**
- If both the injections are given intravenously, the **animal dies in 12–24 hours after second injection**. Autopsy examination shows bilateral cortical necrosis of the kidneys and patchy haemorrhagic necrosis in the liver, spleen and other organs
- Similar mechanism may be responsible for some clinical conditions such as purpuric rashes of meningococcal septicaemia (Waterhouse-Friderichsen syndrome) and dengue haemorrhagic fever

25

Chapter

Autoimmunity

■ Write an explanatory note on autoimmunity.

The recognition of self and nonself is an important function of immune system. It is the fundamental characteristic of immune system to prevent response to self as antigen by appropriate mechanisms under normal circumstances. Under some circumstances, these mechanisms might break down leading to immunological response to self-antigens by treating them as foreign antigens. This abnormal immune response, in which antibodies or sensitized lymphocytes capable of reacting with self-components are produced is known as autoimmunity. The autoimmune response may lead to autoimmune disease. Thus, autoimmune disease is a condition in which the 'natural unresponsiveness' or 'tolerance to self' terminates, as a result of which antibody or sensitized lymphocyte reacts with self-antigen-causing disease.

■ Discuss various mechanisms involved in the development of autoimmune diseases.

Different theories have been proposed to explain how the normal controlling mechanism fails to maintain self-recognition. These are described below.

Hidden or Sequestered Antigen Theory

- According to this theory, the tissue antigens that are exposed to the lymphoreticular system during embryonic development are recognized as **self-antigens** and hence are unable to induce immune response. However, many body antigens (autoantigens) are normally not accessible to the cells of immune system because they are hidden within a cell or tissue, e.g. eye lens protein, brain tissue protein, thyroglobulin and sperm have no opportunity to develop tolerance because they are anatomically confined to sites that prevent their access to lymphoreticular system.
- These antigens are known as **sequestered antigens** and though they are self-antigens, treated as nonself antigens.
- Contact of these antigens with immunocompetent cells results in immune response and at the same time allows the stimulated lymphocytes and antibodies to produce trauma or injury by reacting with them.
- Spermatozoa have no opportunity to develop tolerance because they acquire antigenicity during maturation; hence they are treated as nonself and their exposure to immunocompetent cells lead to orchitis.
- Thus, exposure of the sequestered tissue antigens to the cells of immune system through infection or trauma in latter life lead to autoimmune disease.

Altered Forms of Self-antigens or Neoantigens

In certain circumstances, a native tissue antigen may be altered in some way by physical (irradiation), chemical (drugs and other chemicals) or biological (viral infections, microbial

enzymes) means and thus assume a **new antigenic specificity**. This altered antigen stimulates an immune reaction, which damages native tissue antigen producing tissue injury.

Shared or Cross-reacting Antigens

- Some microorganisms carry **antigenic determinants that resemble the host cell components**. These are known as cross-reacting antigens. Infection with such microorganisms may initiate an immune response that damages particular organ or tissue in the host, e.g. in rheumatic heart disease, antibodies produced against Group A streptococci cross-react with human heart tissue and produce injury and the neurological injury following antirabies vaccine prepared by using sheep brain tissue because of sharing of antigens between human and sheep brain.

Loss of Immunoregulation

The **loss of normal tolerance control** because of loss of immunoregulation leads to autoimmune disease. The defect or lack of efficiency in the lymphoid cell population may result in loss of normal tolerance control, e.g.

- Functional loss of activity of T helper cells (enhanced Th activity) and T suppressor cells (decreased Ts activity)
- Defects in the development of stem cells in the thymus
- Nonspecific polyclonal B-cell activation by certain stimuli such as 2-mercaptoethanol, lipopolysaccharide, trypsin, nystatin, Epstein-Barr virus, and malarial parasites have also been postulated as causes

Genetic Abnormalities

The genetic abnormalities such as mutation of immunocompetent cell, defect in IR (immune response) genes or immunoglobulin gene may lead to **antigenic responsiveness to self-antigens** causing autoimmune diseases.

A disordered immune regulation based upon genetically determined imbalances of the T helper/inducers and T suppressor/cytotoxic is an important determinant in the development of autoimmune diseases as per recent evidence.

■ Classify autoimmune diseases.

Based on the site of involvement and nature of lesions following four types of autoimmune diseases are known:

1. Haemolytic autoimmune diseases
2. Organ-specific or localized autoimmune diseases
3. Nonorgan-specific or systemic autoimmune diseases
4. Transitory diseases

■ Describe haemolytic autoimmune diseases.

Haemolytic autoimmune diseases include:

Autoimmune Haemolytic Anaemias

Two types of autoantibodies against RBCs are produced:

1. Cold antibodies—complete, agglutinating IgM type that agglutinate RBCs at 4°C
2. Warm antibodies—incomplete, nonagglutinating, IgG type that coat the surface of RBCs and cause premature lysis in spleen and liver leading to anaemia. Complement-dependent intravascular haemolysis may also occur

Autoimmune Thrombocytopenia

Autoantibodies against platelets are produced which cause decrease in the number of platelets, e.g. idiopathic thrombocytopenic purpura

Autoimmune Leucopenia

Abs against leucocytes are produced which cause lysis of leucocytes, e.g. nonagglutinating antileucocyte antibodies causing lymphocytopenia in SLE and rheumatoid arthritis

■ **What are organ-specific or localized autoimmune diseases? Describe some organ-specific autoimmune diseases.**

Autoimmune diseases affecting a specific organ are known as organ-specific or localized diseases. These include:

Hashimoto's Disease (Lymphadenoid Goitre)

Cytotoxic autoantibodies are produced which react with thyroglobulin—a thyroid cell component—causing enlargement of thyroid gland and symptoms of hypothyroidism or frank myxedema. The disease is frequently seen in females.

Grave's Disease (Thyrotoxicosis)

An IgG type of antibody known as long-acting thyroid stimulator (LATS) against thyroid membrane antigen is produced, which stimulates excessive hormone secretion. Excessive hormone secretion stimulates cell division leading to enlargement of thyroid

Autoimmune Orchitis

Sperm agglutinating antibodies causing aggregation of spermatozoa are produced, which interfere with penetration of sperm into cervical mucosa. It occurs as a rare complication of mumps infection, in which the virus damages testes allowing entry of the cells of immune system to initiate an immune response.

Myasthenia Gravis

In this disease, there is an abnormal fatigability of muscles due to malfunction of the myoneural junction. Autoantibodies to muscle acetylcholine receptors are produced, which prevent combination of acetylcholine with its receptor and impairs muscular contraction.

Autoimmune Diseases of the Eye

The following two types of autoimmune diseases of eye occur:

1. Phacoanaphylaxis—intraocular inflammation due to autoimmune response to the lens protein following the cataract surgery
2. Perforating injuries—involving the iris or ciliary bodies are followed by the sympathetic ophthalmia in the opposite eye

Pernicious Anaemia

The following two types of antibodies are produced:

1. One is directed against parietal cells of gastric mucosa. It is believed to cause achlorhydria and atrophic gastritis

2. The second antibody is directed against the intrinsic factor and prevents absorption of Vitamin B₁₂. The result is megaloblastic anaemia.

Autoimmune Diseases of the Nervous System

- Neuroparalytic accidents following rabies vaccination due to cross-reaction between human and sheep brain antigens
- Idiopathic polyneuritis (Guillain-Barré syndrome) is considered to be an autoimmune response against peripheral nervous system

■ What are nonorgan-specific (systemic) autoimmune diseases? Describe some nonorgan-specific autoimmune diseases.

Diseases involving several organs or tissue systems are known as nonorgan-specific or systemic autoimmune diseases.

Systemic Lupus Erythematosus (SLE)

- It is a chronic generalized disorder involving many organs/systems with remissions and exacerbations
- It is mainly seen in females and characterized by a red rash across the nose and upper cheeks, which is why the name is lupus erythematosus (LE)
- Haemolytic anaemia, leucopenia, thrombocytopenia; lesions in kidney, vascular tissues, joints, spleen and heart are the other signs and symptoms
- A variety of autoantibodies directed against cell nuclei, intracytoplasmic cell constituents, thyroid, RBCs, WBCs and other unknown antigens are produced
- The important immunological feature is LE cell phenomenon. The LE cell is a polymorphonuclear leucocyte with ingested nuclear material complexed with antinuclear antibody
- Test for the presence of these cells may be used to verify the diagnosis of SLE by incubating patients' blood or bone marrow at 37°C and then observing for LE cells

Rheumatoid Arthritis (RA)

- It is an inflammatory disease of joints and connective tissue commonly associated with serositis, myocarditis, and vasculitis and other disseminated lesions
- The synovial membranes of affected joints are swollen and oedematous with dense infiltration of lymphocytes and plasma cells
- The striking feature is the presence of a circulating autoantibody known as rheumatoid factor (RF)—an IgM that reacts with IgG *in vitro* is found in the sera and synovial fluid of most adult patients
- Other antiglobulins of the IgG, IgA and IgE class are also seen
- RA factor test is used for diagnosis but it is not specific as RF is also found in other diseases, including the connective tissue disorders

Sjögren's Syndrome (SS)

It is a triad of keratoconjunctivitis sicca (dry eyes), xerostomia (dryness of mouth) with or without salivary gland enlargement and rheumatoid arthritis. Patient shows a variety of antibodies—antinuclear antibodies, RF, antibodies against salivary duct, lacrimal glands, smooth muscle, mitochondria, thyroid gland, etc.

Polyarteritis Nodosa

It is a necrotizing angiitis involving medium and small arteries. The disease ends fatally due to coronary thrombosis, cerebral haemorrhage or gastrointestinal bleeding

■ What are transitory diseases?

- Transitory diseases are the conditions such as anaemia, thrombocytopenia or nephritis that occur following certain infections with microbes or drug therapy
- The infecting agent or drug induces antigenic alteration in some self-antigens that initiate an immune response leading to tissue damage
- The disease is transient and undergoes spontaneous cure when the infection is brought under control or the drug is withdrawn

26

Chapter

Transplantation and Tumour Immunity

■ Why is transplantation performed? How successful is this procedure?

- When any organ or tissue is damaged because of disease or injury, or when an organ is congenitally defective or absent, a transplantation or grafting becomes necessary for restoration of function
- Transplantation of certain organs is a very common procedure but high percentage of grafts are unsuccessful due to immunological response of individual to foreign histocompatibility Ags of the donor

■ Define the following terms: Graft/transplant, donor, recipient, living donor graft and cadaveric donor graft.

- **Graft/transplant:** Tissue or organ transplanted is known as graft or transplant
- **Donor:** Individual from whom transplant is obtained is termed donor
- **Recipient:** Individual on whom graft is applied is termed recipient
- **Living donor graft:** When the graft is obtained from a living donor (in case of duplicated organs such as kidneys) it is termed living donor graft
- **Cadaveric donor graft:** When a graft is obtained from a recently deceased individual (as is necessary in the case of an essential organ such as heart or liver) it is termed cadaveric donor graft

■ Mention the different types of transplants.

The different types of transplants are described below:

- Based on the organ/tissue transplanted—kidney, heart or liver transplant
- Based on anatomical site of origin and site of its placement. These are of two types:
 1. **Orthotopic:** Grafts placed in anatomically normal sites, e.g. skin grafting
 2. **Heterotopic:** Grafts placed in anatomically abnormal sites, e.g. thyroid tissue transplanted in a subcutaneous pocket
- Transplants may be fresh or stored tissues or organs
- Transplants may be living or dead:
 - Living: Heart, kidney, liver—vital grafts
 - Nonliving: Bone, artery, etc.—static or structural grafts
- Based on genetic and antigenic relationship of the donor and recipient. These are of four types:
 1. **Autograft (autogenic graft):** A graft taken from one site and transplanted back onto the same individual at another site, e.g. skin transplanted from the thigh to the chest in a patient with severe burns
 2. **Isograft (syngeneic graft):** A graft taken from one individual and placed on another individual of the same genetic constitution, e.g. grafts between identical twins or highly inbred strains of syngeneic animals

3. **Allograft (homograft):** A graft taken from one individual and placed on genetically nonidentical member of the same species. The majority of grafts are allografts
4. **Xenograft(heterograft):** Grafts between members of different species. The xenograft is not often used, e.g. a baboon liver transplanted into a human with liver failure, pig to man, horse to man, etc.

■ **What is rejection? How can it be confirmed that rejection of a transplant is an immunological event?**

- Rejection is the process by which the immune system of the host recognizes, becomes sensitized against Ags of graft and attempts to eliminate the antigenic differences of the donor organ. Except for the autografts and isografts, some degree of rejection occurs with every transplant
- Immunological basis of rejection is evidenced through following sets of reactions:

1. Primary Rejection (First Set Reaction)

- When a graft from an animal is applied on genetically unrelated animal of same species, the graft appears to be accepted initially. It becomes vascularized, appears functionally and morphologically healthy for first 2 or 3 days
- Between 3 and 9 days, it is invaded by lymphocytes and macrophages. Blood supply to graft is diminished and graft undergoes ischaemic necrosis and sloughs off between 10 and 13 days
- During this, the host encounters the histocompatibility Ags of the transplant for the first time. These antigens are processed by macrophages locally or within the regional lymph nodes to sensitize them to initiate immune injury—either the cell-mediated or humoral immune response.

2. Second Set Reaction

- In an animal, which has already rejected graft by first set reaction when another graft is applied from the same donor, rejection occurs rapidly
- Vascularization occurs but is immediately interrupted by the inflammatory response
- Necrosis sets early and the graft sloughs off within 1 week
- This is because of previous sensitization of recipient to the histocompatibility Ags of the graft during first set reaction
- This rapid rejection of the same type of graft indicates that the lymphoid cells are primed and retain memory of the first contact with graft rejection

■ **Explain the mechanisms of graft rejection.**

Graft rejection is mediated through lymphocytes and/or antibodies.

Lymphocyte-mediated Rejection

- Transplantation immunity is predominantly cell mediated
- T-lymphocytes play primary role in first set response and in shortening the graft survival. They recognize target cells through specific surface receptors and combine with Ag, causing surface membrane changes responsible for cytotoxic activity of lymphocytes
- Immunological injury may be mediated in various ways, viz.
 - Directly by cytotoxic T-cells
 - Directly by natural killer cells
 - Indirectly by releasing soluble T-cell mediators of immune injury (lymphokines), or
 - By Ab-dependent cellular cytotoxic (ADCC) attack by killer (K) cells on the target organ

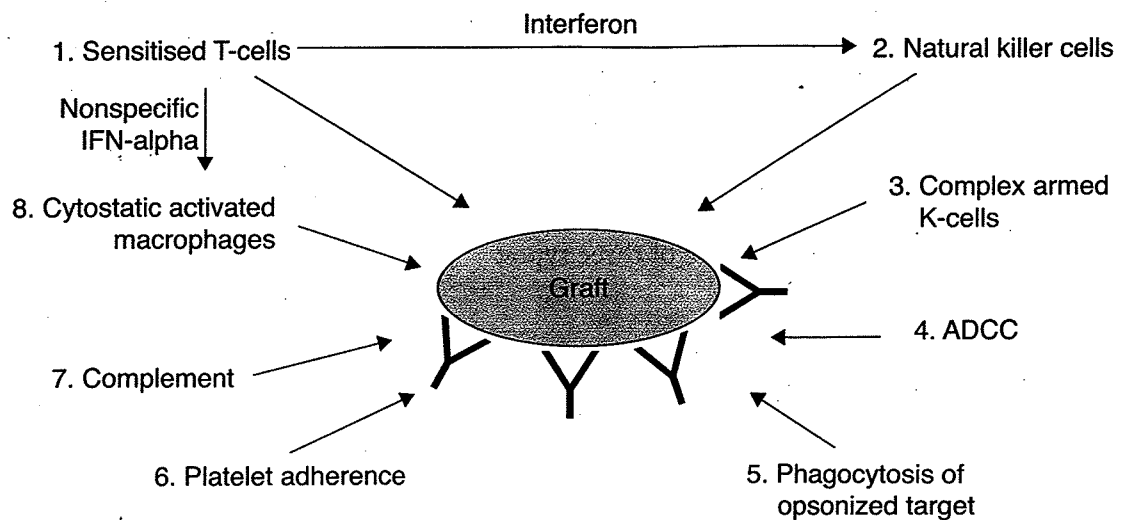


Fig. 26.1 Mechanism of graft rejection.

The Role of Antibodies

- Abs are formed more rapidly and abundantly during the second set response and play an important role along with CMI in a second set reaction
- Antibodies get promptly deposited along the vascular endothelium and activate the complement and coagulation system, which results into deposition of fibrin, infiltration of site with polymorphonuclear leucocyte, platelet thrombosis and prompt coagulative necrosis
- Abs also help killer cells (K) to attack on target organ (ADCC)
- When graft is applied to recipient possessing a high titre of specific Abs, hyperacute rejection occurs. The graft remains pale and is rejected immediately, within hours. This is known as the “**white graft response**”. Hyperacute rejection in humans may occur sometimes following kidney transplant due to pre-existing Abs in recipient as a result of prior transplantation, transfusion or pregnancy

The mechanism of graft rejection can be summarized as (Fig. 26.1):

1. Direct killing by sensitized T-cells; Indirect tissue damage by releasing lymphokines
2. Killing by NK cells—activity enhanced by interferon
3. Killing by immune-complexed armed cells, which recognize the target through free Ab in the complex
4. ADCC attack
5. Opsonization—phagocytosis of the target coated with Ab with or without complement
6. Platelet adherence to Ab on target, leading to formation of microthrombi
7. Complement-mediated cytotoxicity
8. Attack by nonspecifically activated macrophages by agents such as BCG, endotoxin

■ What measures should be taken to prevent graft rejection?

Graft rejection can be prevented by the following methods:

1. Donor–recipient Tissue Matching

- Immunity against transplant is due to presence of Ags in grafted tissue, which are absent in the recipient. If the recipient possesses all Ags present in donor tissue—there is no immune response and no graft rejection even when donor and recipient are not syngeneic
- Ags responsible for rejection are known as transplantation Ags or histocompatibility Ags. Matching donor and recipient at HLA loci can minimize graft rejection. This matching is

known as **histocompatibility testing**. It can be performed by microcytotoxicity test and mixed lymphocyte reaction

2. Use of Immunosuppressive Agents

- The graft rejection can be minimized, delayed or avoided by using immunosuppressive agents, which interfere with the induction or expression of immune response
- The immunosuppressive agents are of three types:

A. Anti-inflammatory Agents

- The major anti-inflammatory agents are the adrenocortical steroids
- These agents provide broad, nonspecific anti-inflammatory action
- They cause depletion of lymphocytes from the blood and lymphoid organs

B. Antimetabolites

- These agents interfere with the synthesis of DNA, RNA or both and thus inhibit cell division and differentiation
- A number of agents with antimetabolite properties have been used for immunosuppression. These include:
 - The purine antagonists, e.g. azathioprine, 6-mercaptopurine—competitively inhibit purine nucleotide synthesis that results into faulty RNA synthesis
 - The folic acid antagonists, e.g. methotrexate, methopterin—inhibit the action of an enzyme, folic acid reductase
 - The alkylating agents, e.g. cyclophosphamide, chlorambucil—induce breaks in the cross linkage of the DNA helix with faulty re-linkage and subsequent cell death

C. Cytotoxic Drugs

- These include—X-irradiation total body, local to the graft, or extracorporeal irradiation has cytolytic activity, especially on rapidly reproducing cells, e.g. lymphoid cells undergoing sensitization
- Antilymphocyte serum against thymocytes/lymphocytes causes immune cytolysis of lymphocytes
- Agents such as steroids, azathioprine, alkylating agents and actinomycin D

Cyclosporine

- Most powerful agent produced by fungus
- Most widely used cyclic polypeptide
- Mechanism—not exactly known, may act by increasing the activity of T suppressor cells or by inhibiting T helper cell activity. It is not cytotoxic for lymphocytes

3. Lymphoid Cell Ablation

- Removal of lymphoid cells by extracorporeal irradiation of blood and thoracic duct cannulation are helpful in depressing immunological reactivity nonspecifically and increasing the graft survival
- Injections of antilymphocyte serum or monoclonal anti-T3 Abs successfully reverse acute kidney graft

4. Immunological Enhancement

- Under certain circumstances, Abs produced against graft Ags act in opposition to CMI and protect the graft from CMI by inhibiting graft rejection
- This phenomenon is known as “immunological enhancement”
- This happens when the recipient is immunized with one or more injections of killed donor tissue and then transplant is applied subsequently, the graft survival is increased

- These Abs may combine with
 - Ags released from the graft and hence unable to initiate an immunological response
 - Lymphoid cells of appropriate specificity and hence render them incapable of responding to graft Ags
 - They may coat the surface of cells in graft and hence the sensitized lymphocytes are kept out of contact with them

■ Describe graft versus host (GVH) reaction.

- The graft rejection is due to immunological response against graft antigens in the grafted tissue, which are recognized and treated as foreign Ags by the immune system of the host. This is known as host versus graft reaction
- The reverse of this may also occur, in which the graft may mount an immunological response against the Ags of the host. This is known as the graft versus host (GVH) reaction
- This occurs when:
 - The graft contains significant number of T-cells
 - The recipient possesses Ags that are absent in the grafted tissue
 - The recipient must not reject the graft
 - The recipient is generally immunodeficient. The immunocompetent cells in grafted tissue get established in the host and recognize host cell Ags as foreign and reject the host
- The GVH reaction is predominantly CMI
- In animals, it is manifested as fever, anaemia, rash, splenomegaly, weight loss and death
- In neonatally thymectomized or immature animal, retardation of growth, emaciation, diarrhoea, hepatosplenomegaly, anaemia, lymphoid atrophy and death. This condition is known as **runt disease**.

■ How does a tumour induce an immune response? Describe the different types of tumour antigens.

Tumour is a swelling or enlargement, especially one due to pathologic overgrowth of tissue. When cell undergoes malignant transformation it expresses new surface Ags, which make tumour antigenically different from normal tissue of the host. These new Ags are recognized as 'non-self' and hence a tumour behaves as an allograft and induces an immune response.

Tumour Antigens

These are of three types:

1. Antigens Induced by Chemicals

Chemically-induced tumours express different Ags, which are different for each tumour and are not antigenically constant from specimen to specimen.

2. Antigens Induced by Viruses

- These Ags are constant from specimen to specimen
- The viruses such as herpes simplex viruses, adenoviruses, Papovavirus, hepatitis B virus, human T-cell leukaemia virus, Rous sarcoma virus, etc. can induce antigens
- These are tumour associated transplantation Ags (TATAs). They are of two types:
 - Shared tumour Ags are Ags shared by many tumours
 - Specific tumour Ags are Ags specific to an individual tumour

Specific Tumour Ags

These are of three types:

- i. V antigen—associated with infective virion
- ii. Tumour (T) or nuclear Ag—are located in the nucleus of infected tumour cells and are specific for tumour inducing virus

- iii. Tumour-specific transplantation antigens (TSTAs)—are cytoplasmic membrane Ags and are important as these come in contact with immune system. They are present on all tumours induced by specific virus in different animal species

3. Carcinofetal Antigens

Certain tumours in adults show presence of Ags present in fetal or embryonic cells, which are absent in normal adult cells. These include:

- Carcinoembryonic Ag—found in human embryonic gut and gut-associated organs during embryonic life. It is an important marker of cancer of gut in adults
- Alpha-fetoprotein—normally present in fetal serum in high concentration. It is an important marker of hepatoma in adults

■ Write a short note on immune response in malignancy.

Specific Immune Response

- Immune response to tumour is primarily cell mediated
- T-cells play an important role in destruction of tumour cells
- Ags shed by neoplastic cells are presented to T-cells by macrophages (Ag-presenting cells)
- Antigenically stimulated T-cell releases lymphokines
- Activated T-lymphocytes cooperate to B-cells and activate B-cells to produce Abs, which play an important role in destruction of tumour cells
- Activated T-cells and specific Abs have cytostatic or cytolytic activity

Destruction of Tumour Cells

T-cells have cytotoxic activity; Ab-dependent cell cytotoxicity with the help of antitumour Ab and lymphokine-mediated activity.

Nonspecific Immune Response

- Activated macrophages and NK cells nonspecifically destroy tumour cells
- Armed macrophages bind nonspecifically with IgG or IgM Ab by Fc receptor and become nonspecifically activated against tumour cell and produce cytolytic effect

■ How is immunological surveillance built and maintained? Also give reasons for its lapse.

- Immunological surveillance is based on the concept that tumour cells display Ags, which are treated as non-self by the host's immune system and induce an immune response that destroys transformed cell
- Thus, immune system acts as an active guardian against transformed cells carrying foreign or non-self Ags
- It is postulated that the immune system keeps a constant vigilance on transformed cells that arise by mutation of somatic cells and destroy them
- Development of tumour represents an escape from the surveillance mechanism according to this hypothesis
- Insufficiency of the surveillance mechanism due to ageing or congenital or iatrogenic immunodeficiencies, leads to an increased incidence of cancer
- The development of tumours represents a lapse in surveillance. This may be due to fast rate of proliferation of malignant cells because of which they may escape before the development of an effective immune response
- Circulating tumour antigens may coat the surface of lymphoid cells and prevent their contact with tumour cells

- Contact of tumour antigens with immunocompetent cells may be prevented because of cover of antigenically neutral substances—no immune response
- Humoral antibodies may cause immunological enhancement
- Some tumours may form cytokines like transforming growth factor- β , which suppresses CMI

■ **Write a short note on immunotherapy of cancer.**

The aim of immunotherapy is to augment antitumour defenses. It is of two types:

1. Nonspecific active Immunotherapy

- This uses adjuvant therapy, e.g. immunization with BCG vaccine to boost the nonspecific immune response. BCG potentiates tumour immunity by:
 - Enhancing macrophage cytotoxicity
 - Stimulating T-lymphocyte trapping
 - Activating T-lymphocytes
 - Influencing B-lymphocytes
- Nonliving *Corynebacterium parvum*, glycan—a glucose polymer derived from microorganisms, levamisole—an antihelminthic drug and dinitrochlorobenzene have been found to be effective in stimulating CMI and macrophage function. These agents have been used in different types of cancers with some degree of success

2. Specific Immunotherapy

This includes passive, active and adoptive immunotherapy.

A. Passive immunotherapy

Passive administration of serum containing a high titre of Abs to tumour-associated Ags.

B. Active immunotherapy

By injecting tumour cell vaccines. Presently, vaccines prepared by using purified tumour cell antigen and tumour cells.

C. Adoptive immunotherapy

- Specific adoptive immunotherapy with the help of lymphocytes, transfer factor and immune RNA from persons who have been cured of their neoplasms or specifically immunized against the patients' tumour have been found to boost immunity
- Lymphokine activated killer (LAK) cells obtained by treatment of NK cells with interleukin have been found useful in the treatment, and also in preventing metastases of tumours
- Thymosin, a hormone extracted from bovine or human thymus, has been recently reported to have antitumour effects

27

Chapter

Immunization, Immunizing Agents, Immunoprophylaxis and Immunotherapy

■ What is immunization?

- Immunization is the method by which artificial immunity, i.e. resistance to a particular infection in an individual, is developed artificially by giving immunizing agents
- Immunization methods for the prophylaxis and treatment of diseases in certain situations are the most effective methods for increasing host resistance and ultimately to control certain common and serious infectious diseases. These methods are also useful in noninfectious diseases such as cancer

■ Why is immunization necessary?

Immunization is necessary for the following reasons:

- To provide protection against infectious diseases in children by routine immunization, which forms the part of basic health care system of communities
- To raise overall level of immunity in community (Herd immunity) to control infections in community in addition to providing individual protection
- To provide protection in selected groups or individuals having risk of particular infection (risk groups) by immunizing them against that particular infection

■ Name the immunizing agents used for immunization.

Immunizing agents may be classified as:

- Vaccines
- Immunoglobulins
- Antisera

■ Define vaccine.

- Vaccine is any biological product prepared from microorganisms or other biological substances, e.g. allergens or tumour products, used in prevention, amelioration or treatment of infectious diseases
- These are substances designed to confer specific immunological protection against diseases
- Vaccines may be prepared from live or killed organisms, toxins, extracted cellular fractions or combination of these to develop active immunity

■ Describe the types of vaccines on the basis of their mode of preparation.

On the basis of their mode of preparation vaccines are of the following types:

1. Live Attenuated Vaccines

- Live vaccines are preparations containing live microorganisms with reduced virulence.
- Live vaccines are prepared by using attenuated strains of microorganisms

- Live vaccines initiate infection without causing disease
- The level of immunity induced is same as in natural infection and immunity lasts for several years
- Booster doses are generally not required but may be necessary sometimes
- Can be administered by the route of natural infection and parenterally

Advantages

1. A single dose is usually sufficient
2. Can be administered by the route of natural infection to induce local immunity
3. Also induce humoral and cell-mediated immunity
4. Induces longer and effective immunity

Disadvantages

1. Risk of reversion of virulence in immunocompromised individuals
2. May cause local and remote complications
3. Organism as it is live may spread from vaccinee to contacts

Killed Vaccines

- Killed vaccines are preparations containing killed or inactivated microorganisms. They are prepared by inactivation of microorganisms by heat, phenol, formalin, etc.
- Killed vaccines do not initiate infection and there is no multiplication of microorganism
- They induce active immunity but the level of immunity induced is less as compared to natural infection and live vaccine, and also it lasts for short duration, hence booster doses are necessary

Advantages

1. Safe and stable
2. Can be given in combination as polyvalent vaccines
3. No danger of spread of organism from vaccinee to contacts

Disadvantages

1. Multiple injections are needed (booster doses)
2. Cannot be administered orally. Oral route is not suitable (effective by parenteral route)
3. Unable to induce local and cell-mediated immunity
4. May sometimes cause complications (hypersensitivity reactions)

Toxoid

Certain microorganisms produce exotoxins, e.g. diphtheria and tetanus bacilli, which play an important role in pathogenesis. These toxins can be detoxified and used for immunization. The detoxified toxin is known as toxoid, which is nontoxic but antigenic. The toxoids are prepared by treating toxin with formalin or heat. Toxoid when injected, produces antitoxin, which specifically reacts with toxin and neutralizes it, e.g. tetanus toxoid for tetanus (TT), diphtheria toxoid for diphtheria (DT).

The antigenicity of toxoid can be potentiated by adjuvant. Alternatively, their activity can be enhanced by mixing them with a bacterial vaccine, e.g. diphtheria pertussis tetanus (DPT) in which pertussis is a vaccine that potentiates the activity of diphtheria and tetanus toxoids.

2. Cellular Fractions

Some vaccines are prepared using extracted cellular fractions. These include:

- Meningococcal vaccine from the antigen of the cell wall
- Pneumococcal vaccine from the capsular polysaccharide
- A purified capsular polysaccharide vaccine for *H. influenzae* type b

3. Subunit Vaccines

In this, detergent or chemical is used to split the microorganism and only the relevant antigens, which are immunogenic components, are used for vaccine preparation. These vaccines include rabies and influenza vaccines

4. Recombinant Vaccines

In this, the genes responsible for specific Ags, which are immunogenic components from organisms difficult to grow *in vitro*, are cloned in organisms like *E. coli*, Vaccinia virus or other suitable vectors and then the antigen is obtained in large amount. The technique is known as **DNA recombinant technology**. These vaccines include hepatitis B and influenza vaccines

5. Combined Vaccines

These are the vaccine preparations containing more than one immunizing agent. They are also known as *mixed vaccines*. These include:

- DPT (Diphtheria, Pertussis, Tetanus)
- DT (Diphtheria, Tetanus)
- DP (Diphtheria, Pertussis)
- MMR (Mumps, Measles, Rubella)
- DPTP (DPT + Polio)

Advantages

1. To simplify administration
2. To reduce costs
3. To minimize number of injections and contact with health system

■ Describe in short the agents used for immunotherapy (passive immunization).

For this purpose, immunoglobulins and antisera are used.

Immunoglobulins

Immunoglobulins are of the following two types:

1. Normal human immunoglobulin
2. Specific human immunoglobulin

Normal Human Immunoglobulin

- It is an antibody-rich fraction derived from blood, plasma or serum of human donors
- It is obtained from a pool of at least thousand donors
- The preparation contains high levels of appropriate antibody mainly IgG

Uses

1. It is used to prevent measles in highly susceptible individuals and to provide temporary protection
2. It is also used against hepatitis A infection for travellers to endemic area and to contacts of cases of hepatitis A in an outbreak
3. Its use has also been suggested in immunodeficiency diseases

Specific Human Immunoglobulins

- These are prepared from serum or plasma of convalescent individuals (patients who have recently recovered from infection) or those who have been hyperimmunized with a specific antigen and are used for passive immunization

- These preparations contain high level of specific antibodies, e.g.
 1. Rabies immunoglobulin
 2. Hepatitis B immunoglobulin
 3. Tetanus immunoglobulin
 4. Pertussis immunoglobulin
 5. Rh (D) immunoglobulin
 6. Varicella-zoster immunoglobulin
 - These are usually given by intramuscular route
 - The average half-life is 20–35 days and protection may last up to 3 months or more
 - Use of this preparation minimizes the risk of hypersensitivity reactions

Antisera and Antitoxins

- The terms antiserum and antitoxin are applied to materials prepared in animals, usually horses. Sheep, goat, rabbit, guinea pigs or human beings are also used.
- The antiserum is prepared by injecting specific antigen that leads to formation of specific antibody, e.g. rabies antiserum
- Antitoxin is prepared by injecting toxin or toxoid, e.g. anti-tetanus serum (ATS), anti-diphtheria serum (ADS), anti-snake venom (ASV), anti-gas gangrene serum (AGS), etc.
- The half-life of antisera is half of the human immunoglobulin and elimination occurs more rapidly than human immunoglobulin
- Antisera and antitoxins are used for passive immunization only in clinical situations in which there is no alternative. This is because they present the problem of possible hypersensitivity reactions such as development of anaphylaxis or serum sickness

UNIT

III

Systemic Bacteriology

28

Chapter

Laboratory Diagnosis of Bacterial Diseases

■ What precautions should be taken while collecting specimen for laboratory diagnosis?

- Specimens should be obtained before starting antibiotic therapy
- They should be collected from the appropriate site
- Specimens should be collected under aseptic conditions
- Sufficient quantity of specimen should be collected so as to allow complete examination
- It must be collected in a sterile container—culture tube or plain sterile bulb
- It should be accurately labelled and accompanied by requisition form with patient's name, registration no., ward, age, and type of specimen, investigations required, clinical diagnosis and clinical details
- The containers should be labelled properly with patient's name, registration no., ward, etc.
- The specimens likely to contain highly infectious organisms should be labelled as 'High Risk' along with warning symbol

■ Give the collection procedure for the following specimens: urine, genital tract, cerebrospinal fluid, stool, sputum, throat swab, serous fluid, pus, and blood.

Collection of Urine

- Give the patient sterile, dry, leak proof container with instructions to collect mid-stream urine sample after cleaning local parts
- The normal flora of anterior urethra is flushed out by passing first portion of urine before collection of specimen of urine for culture
- Subsequent midstream urine is collected in a sterile test tube
- For acid-fast bacteria, three early morning samples on three successive days are collected
- Specimen of urine can also be obtained by catheterization or suprapubic aspiration

Collection of Genital Tract Specimens

1. Urethral Discharge

It may be expressed at anterior urethra and collected with swab or it can be collected directly with loop.

2. Cervical Swab

A sterile speculum—moistened with warm water is used and sterile swab is inserted into endocervical canal, moved gently and left in place for 20–30 seconds for absorption of bacteria on swab.

3. Vaginal Swab

It is collected from posterior vault or vaginal orifice.

Collection of Cerebrospinal Fluid (CSF)

By taking all aseptic precautions, lumbar puncture is done and CSF is collected in a sterile container. If delay is expected then CSF is collected into glucose broth and incubated at 37°C. CSF should never be refrigerated to avoid death of some organisms at freezing temperature.

Collection of Stool

- Give the patient dry, leakproof container with instructions to collect stool sample and portion of it containing mucus, pus, or blood is transferred to suitable transport medium
- If stool sample is not available, a rectal swab can be collected by inserting cotton swab into rectum for about 10 seconds
- Swab from ulcer is collected by sigmoidoscopic examination

Collection of Sputum

- Give the patient dry, leakproof, wide-mouth container with instructions to collect sample by deep coughing
- Sample must be sputum and not saliva
- A morning sample of sputum is ideal
- If tuberculosis is suspected, morning specimen should be collected on three successive days

Collection of Throat Swab

- Patient is asked to open the mouth
- Tongue is depressed by using tongue depressor
- Swab is then rubbed firmly over the back of the throat (posterior pharynx), tonsils, tonsillar fossae and other areas of inflammation, exudation or ulceration
- Care should be taken to avoid touching swab with tongue, cheeks and lips to avoid contamination
- The swab is placed in sterile test tube and sterile saline is added to prevent drying

Collection of Serous Fluids

- Synovial fluid is collected by aspiration
- Ascitic and pleural fluids are collected by tapping
- Fluids must be collected in a sterile container with citrate to prevent clotting

Collection of Pus

Pus can be collected by aspiration from abscess or it can be collected with the help of sterile cotton swab from infected tissue, if it is scanty.

Collection of Blood

- By taking all aseptic precautions, 10 ml of blood is collected by venepuncture
- 5 ml of blood is added to 50 ml of transport medium to dilute it tenfold to inhibit bactericidal effect of blood
- In infants and children, 1–5 ml of blood is sufficient

- Blood can be collected into medium with sodium polyanethol sulfonate, which helps in preventing clotting of blood and also neutralizes natural bactericidal substances and some antibiotics in blood

■ **What negative effects would delay in transport have on specimens collected for laboratory diagnosis? How can this be avoided? Cite examples.**

- Delay in transportation to laboratory may cause:
 - Death of delicate organisms
 - Overgrowth of other organisms
- Specimen should be transported immediately
- If delay is inevitable, specimens should be collected in suitable transport media or in a special container to avoid death during transport
- Specimens should be transported in leakproof containers as per the safety guidelines

Stool

- Transported immediately. If a delay of more than 2–4 hours is expected then it should be collected in a suitable transport medium such as Gram-negative broth, Cary–Blair transport medium and buffered glycerol saline

Swabs

If delay is expected, the swab should be collected in a suitable transport medium such as Stuart's medium or Amies transport medium.

Blood

It is collected and transported in glucose broth, bile broth, thioglycollate broth or brain–heart infusion broth.

Urine

- Urine should reach the laboratory within 1 hour of collection; if not possible, it should be refrigerated at 4°C
- If delay of more than 1 hour is expected then 0.1 g of boric acid powder per 10 ml of urine is added

CSF

If delay is expected, CSF should be added to glucose broth and incubated.

Sputum

- Sputum for culture should reach laboratory within 2 hours of collection
- It can be refrigerated up to 24 hours, or cetylpyridinium chloride–sodium chloride (CPC–NaCl) is added in equal volume if more delay is expected
- CPC–NaCl digests sputum and maintain viability of mycobacteria for 8 days and slows down the growth of commensals

■ **By which methods of direct microscopy can microorganisms be identified/detected?**

Direct microscopy methods used for identification/detection of microorganisms are given below:

Wet Mount

It is used for liquid specimens to observe presence of cells and bacteria.

Hanging Drop Preparation for Motility

It is used for presumptive diagnosis of cholera.

Gram Stain

- Gram stain is performed routinely,
 - To reveal the shape and size of bacteria
 - To classify organisms into four categories:
 1. Gram-positive cocci
 2. Gram-positive bacilli
 3. Gram-negative cocci
 4. Gram-negative bacilli
- Gram stain gives clue to plan antibiotic therapy

Ziehl-Neelsen's Stain

Z-N stain is performed on specimens, including sputum, urine, pus, etc. when infections by mycobacteria or *Nocardia* are suspected.

Direct Immunofluorescence

In this method smears are treated with antibody labelled with fluorescent dye, and specific antigen can be detected by using fluorescent microscope.

Other Special Staining Techniques

A number of other techniques can be used for identification of different bacteria. These include:

1. Fluorescence microscopy for tubercle bacilli
2. Dark ground microscopy for *Treponema pallidum*
3. India ink (negative staining) for capsulated bacteria
4. Albert's and Neisser's stain for *C. diphtheriae*
5. Spore staining for sporulating bacteria
6. Other staining methods according to the bacterium suspected

■ **Mention the cultural conditions suitable for growth of medically important bacteria.**

- Culture is the most reliable method for confirming the diagnosis
- Most of the medically important bacteria grow *in vitro* on artificial media

Growth Requirements

- pH 7.2–7.4
- Temperature 35°–37°C
- Incubation period—12–18 hours (up to 48–72 hours)

Growth Conditions

- Aerobic or capnophilic (5–10% CO₂) or anaerobic, as required

Media for Culture

- Routinely specimen is inoculated on blood agar and MacConkey agar
- If required, selective media are used

These include:

- Chocolate agar—for *H. influenzae*, *Neisseria* spp.
- Deoxycholate citrate agar—for *Salmonella* and *Shigella* spp.

- TCBS agar—for *V. cholerae*
- Wilson and Blair's medium—for *Salmonella* spp.
- Thayer–Martin medium—for *Neisseria* spp.
- Lowenstein–Jensen medium—for mycobacteria
- Robertson's cooked meat medium—for anaerobes

■ **Enumerate the methods by which microorganisms grown in a culture medium can be identified.**

The organisms grown in culture are identified on the basis of:

- Growth characteristics, e.g. aerobic or anaerobic
- Colony morphology—helps in presumptive identification of most of the pathogenic bacteria
- Microscopic examination of colony smear
- Biochemical tests such as:
 - Sugar fermentation reactions
 - Indole production
 - Methyl red test
 - Voges–Proskauer test
 - Citrate utilization test
 - H₂S production
 - Urease test
 - Oxidase test
 - Catalase test
 - Phenylalanine deaminase test
 - Nitrate reduction test
 - Gelatin liquefaction
 - Amino acid decarboxylase test

■ **Why is antimicrobial susceptibility testing important?**

Susceptibility testing to different antimicrobial agents is important to institute rational antimicrobial therapy. This is very important to guide the therapy, particularly in cases not responding to routine antibiotic therapy or in infections caused by drug resistant pathogens. Susceptibility testing methods used commonly are:

- Disc diffusion method
- Tube dilution method

Kirby–Bauer disc diffusion method is the most common and widely used method.

■ **Why and on what basis are serological tests performed? Name the commonly used serological tests.**

- Serological tests are commonly used in the diagnosis of infections in which causative agent is difficult to culture, e.g. *Treponema pallidum*, *Mycoplasma pneumoniae*, *Chlamydiae*, etc.
- Diagnosis is done on the basis of detection of specific antibody or antigen to the infectious agent
- Active infection is diagnosed by demonstrating specific IgM or a fourfold increase in IgG antibodies in paired sera taken 10–14 days apart

The commonly used serological tests in different bacterial infections are:

- Widal test in enteric fever
- Brucella agglutination test in brucellosis
- Indirect Coombs test—for demonstration of incomplete antibodies

- VDRL test—for syphilis
- Enzyme-linked immunosorbent assay (ELISA)
- Radioimmunoassay (RIA) test
- Indirect immunofluorescence test—for syphilis, *Legionella*, *Mycoplasma*, *Borrelia* and some other infections

■ How are bacterial antigens detected?

Bacterial antigens can be detected by

- **Latex agglutination test**—in infections due to *H. influenzae*, streptococci, *Neisseria*, etc.
- **Co-agglutination test**—in streptococcal, pneumococcal, neisserial, *Salmonella*, *H. influenzae* and some other infections
- **Enzyme-linked immunosorbent assay test (ELISA)**—in various bacterial infections
- **Radioimmunoassay (RIA)**—in various bacterial infections
- **Slide agglutination test** is also used for identification and confirmation of bacteria grown in culture

■ List the molecular methods for detection of microorganisms. In 2–5 sentences explain any two methods.

- The following methods are used for detection of nucleic acid:
 - Nucleic acid probe
 - Polymerase chain reactions (PCR)
 - Ligase chain reactions (LCR)
 - Nucleic acid sequence based amplification (NASBA)
 - Branched DNA technique
 - Strand displacement amplification (SDA)
- **Nucleic acid probe** uses gene probes (cloned fragments of DNA) that recognize complementary sequences of nucleic acid of microorganisms. Based on this the infecting organism is identified. The test can be used for diagnosis of mycobacterial, chlamydial, neisserial and other bacterial infections
- **Polymerase chain reaction** uses the ability of DNA polymerase to synthesize a large amount of specific DNA from a single piece of DNA in the sample or target DNA being sought. PCR can be used for the diagnosis of chlamydial, neisserial, mycobacterial and other bacterial infections
 - PCR is highly sensitive and is particularly important in the identification of organisms that are difficult or slow to grow

(Other enlisted methods of nucleic acid detection are not explained as they are not included in the syllabus.)

■ Besides culture, bacterial antigens and molecular detection, which other methods are used for identification of microorganisms?

Other methods for identification include:

- Antigenic characters
- Growth factor requirements
- Toxin production
- Metabolic end products by gas liquid chromatography
- Enzyme production

■ **Under which circumstances does typing of a strain becomes necessary? List the methods by which it can be performed.**

Identification of bacteria to species level is normally satisfactory but sometimes further characterization of individual isolate may be necessary to trace epidemic spread of an organism. This can be done by

- Antigenic typing, e.g. *E. coli*
- Phage typing, e.g. *Staph. aureus*
- Antibigram/resistogram, e.g. *Klebsiella* and *Pseudomonas*
- Bacteriocin typing, e.g. *Pseudomonas*
- Characterization of chromosomal DNA, e.g. *M. tuberculosis*
- Plasmid analysis, e.g. *E. coli*

29

Chapter

Staphylococcus

■ Name the scientists associated with the discovery of *Staphylococcus*.

- It was first observed by von Recklinghausen in 1871
- It was isolated by Pasteur in 1880
- Causative role in pyogenic infection was proved by Sir Alexander Ogston. The name staphylococcus (staphyle means bunch of grapes, kokkos means a berry in Greek) was also given by him.
- Rosenbach described *Staph. aureus* and *Staph. albus* in 1884 and Passet described *Staph. citreus* in 1885

■ Which species of *Staphylococcus* are important to human beings?

Species of human importance are:

- *Staph. aureus*
- *Staph. epidermidis* (*Staph. albus*)
- *Staph. saprophyticus*
- *Staph. hominis*
- *Staph. haemolyticus*
- *Staph. simulans*

✓ ■ Classify Staphylococci on the basis of coagulase production.

Based on coagulase production *staphylococci* are classified into two groups:

1. Coagulase-positive staphylococci, e.g. *Staph. aureus*
2. Coagulase-negative staphylococci, e.g. *Staph. epidermidis*, *Staph. saprophyticus*

In past, staphylococci were differentiated into three types, based on pigment production (Fig. 29.1):

1. *Staph. aureus*—golden yellow
2. *Staph. citreus*—lemon yellow
3. *Staph. albus*—white

Most pathogenic staphylococci produce golden yellow pigment but association between virulence and pigment production is not constantly seen.

■ Enumerate the important morphological features of *Staph. aureus*.

Morphological Features

- Gram-positive cocci, about 1μ in diameter
- Arranged in clusters—cluster formation is due to cell division occurring in three planes with daughter cells remaining close together
- Nonmotile, nonsporing and noncapsulated (Fig. 29.2)



Fig. 29.1 Three types of Staphylococci.

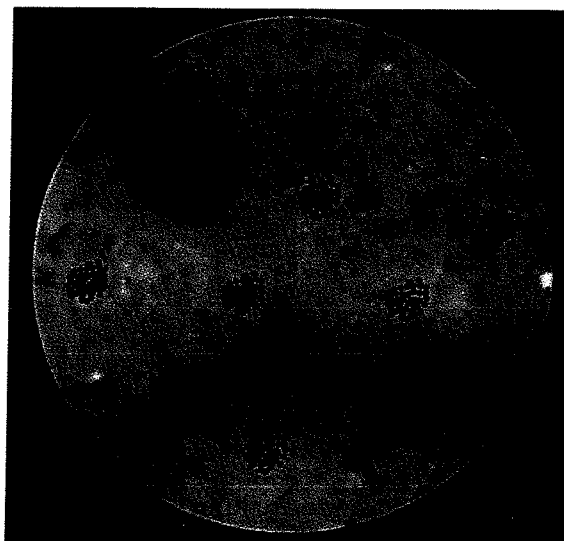


Fig. 29.2 Staphylococci.

■ Give the cultural characteristics of *Staph. aureus*.

Cultural Characters

- Aerobe and facultative anaerobe
- Optimum temperature 37°C (range, 12°C–44°C) and optimum pH 7.5
- Can grow on ordinary media

Media and Colony Characters

- **Nutrient agar**—large, 2–4 mm in diameter, round, smooth, raised, opaque, shiny, easily emulsifiable. Most strains produce golden yellow localized pigment (Fig. 29.1)
- Pigment is lipoprotein allied to carotene. Pigment production is enhanced by
 - Aerobic incubation at 25°C
 - Culture on 1% glycerol monoacetate agar
 - Culture on milk agar
- **Nutrient agar slant**—oil paint appearance
- **Blood agar**—colonies are similar to that of nutrient agar along with beta-haemolytic zone especially on sheep and rabbit blood agar

- **MacConkey's agar**—small lactose-fermenting colonies (pink)
- **Selective media**—staphylococci can tolerate 5–10% sodium chloride, lithium chloride, tellurite and polymyxin so these are used in selective media, e.g.
 - Salt milk agar
 - Ludlam's medium
 - Mannitol salt agar—it is a selective and indicator medium. Mannitol-fermenting staphylococcal colonies are surrounded by yellow zone
- **Enrichment broth**—salt cooked meat medium

■ **List the biochemical reactions characteristic to *Staph. aureus*.**

- Ferments many sugars with acid
- Catalase—positive
- Produces phosphatase
- Liquefies gelatin

■ **What are the characteristic features of *Staph. aureus*?**

Characteristic features of *Staph. aureus* or pathogenic staphylococci:

- Coagulase production
- Deoxyribonucleases production
- Mannitol fermentation
- Beta haemolysis on blood agar
- Golden yellow pigment production
- Gelatin liquefaction
- Phosphatase production
- Tellurite reduction

■ **To which factors is *Staph. aureus* resistant/sensitive?**

Staph. aureus is resistant/sensitive to the following factors:

- Killed by heat at 60°C in 30 minutes; some are killed at 62°C in 30 minutes
- Sensitive to disinfectants such as phenol and aniline dyes, e.g. crystal violet (therefore inhibited on crystal violet blood agar used for streptococci)
- Remains viable for 2–3 months in dry pus
- Resistant to lysozyme and sensitive to lysostaphin
- Penicillin resistant strains are found due to production of penicillinase controlled by plasmid
- Resistance to penicillin and cephalosporins may be due to reduction in affinity of penicillin-binding protein of cell wall with beta-lactam antibiotics. This change is usually chromosomal; resistance also extends to cover other beta-lactamase resistant penicillins such as methicillin and cloxacillin
- Plasmid-borne resistance to erythromycin, tetracycline and aminoglycosides and all other antibiotics, except vancomycin, is detected

■ **Write short on each of the following virulence factors produced by *Staph. aureus*: (a) Antigens, (b) Toxins and (c) Enzymes.**

(a) **Antigens**

- **Capsular polysaccharide**—surrounds cell wall. It inhibits opsonization and phagocytosis
- **Peptidoglycan**—confers rigidity to cell, activates complement and induces release of cytokines
- **Teichoic acid**—antigenic component of cell wall facilitates adhesion of cocci and protect them from complement-mediated opsonization

- **Protein A**—present on cell wall is chemotactic and antiphagocytic and shows anticomplementary effects
 - It is found on 90% strains of *Staph. aureus* (specially Cowan I) and is absent on coagulase-negative staphylococci
 - It binds with Fc terminal of IgG, leaving Fab region free to combine with antigens
 - When suspension of such cells coated with IgG is treated with homologous antigen, Ag combines with free Fab portions of IgG attached to cell causing agglutination. This is known as **coagglutination**
- **Clumping factor**—this is another surface protein called bound coagulase responsible for slide coagulase test. When saline suspension of *Staph. aureus* is mixed on slide with a drop of plasma, the cocci are clumped. It is used as routine test for identification of *Staph. aureus*

❧ Toxins

- **Haemolytic toxins:** Alpha, beta, gamma, delta
 - **Alpha** – It is most important in pathogenicity. It is toxic to macrophages and lysosomes
 - **Beta** – It exhibits hot-cold phenomenon, haemolysis begins at 37°C and evident only on chilling
 - **Gamma** – It is antigenic. Antibody to it are detected in patients with deep seated staphylococcal infections
 - **Delta** – It has effect on red blood cells, white blood cells, macrophage and platelets
- **Leucocidin:** It causes damage to polymorphs and macrophages
- **Epidermolytic toxin:** It is responsible for staphylococcal scalded skin syndrome (SSSS)—an exfoliative skin disease where outer layer of epidermis gets separated from the underlying tissue
- **Enterotoxin**
 - This is responsible for staphylococcal food poisoning
 - Toxin is heat stable withstand 100°C for 10–14 minutes
 - Two-third strains of *Staph. aureus* produce it
 - It has eight antigenic types—A, B, C1, C2, C3, D, E and H
 - It acts on autonomic nervous system to cause illness
 - Tests such as latex agglutination and ELISA are used for its detection
- **Toxic Shock Syndrome Toxin (TSST)**
 - Toxic shock syndrome toxin is responsible for toxic shock syndrome
 - 90% strains producing it are isolated from vagina of patients of toxic shock syndrome associated with menstruation and some from other sites

❧ Enzymes

- **Coagulase**
 - This is an enzyme, which clots human plasma
 - It acts with coagulase-reacting factor present in plasma, binds to prothrombin and converts fibrinogen to fibrin
 - Eight coagulase types have been identified
 - Most human strains produce coagulase A
 - It (free coagulase) differs from bound coagulase in some respects (Table 29.1)
- **Lipases:** They degrade lipid and help in infecting skin and subcutaneous tissue
- **Hyaluronidase:** It breaks down connective tissue by hydrolyzing hyaluronic acid in ground substance and facilitates spread of infection
- **Nucleases:** They degrade nucleic acid
- **Fibrinolysin or staphylokinase:** It has fibrinolytic activity and helps in spread of infection

Table 29.1 Differences between free and bound coagulase

Free coagulase	Bound coagulase (clumping factor)
1. It is secreted free into medium	It is a constituent of cell wall bound to it
2. Requires help of coagulase-reacting factor for action	Action is independent of factor
3. Eight types identified (A-H)	Only one type identified
4. Heat labile	Heat stable

- **Protein receptors:** Staphylococci have these receptors for fibrinogen, IgG and complement component C1q. They facilitate adhesion to host cell tissue
- **Others:** Also produces proteases

■ Describe the methods of typing *Staphylococcus*.

Typing methods are:

1. Bacteriophage Typing

- Staphylococci may be typed on the basis of their susceptibility to bacteriophage
- It is important in epidemiological studies of infection
- Staphylococcal phage reference laboratory is at **Maulana Azad Medical College, New Delhi**
- The commonest phage type prevalent in most parts of India is 52/52A/80/81

2. Serological Typing

- All strains of *Staph. aureus* possess common cell surface Ag factor. Hence, they are agglutinated by any staphylococcal serum
- Antisera against *Staph. aureus* is first treated with heterologous strain to remove common agglutinin
- There are two typing methods:
 1. Cowan's method—sera is absorbed to divide the species into three types—Cowan I, II and III
 2. Slide agglutination methods—based on 30 type specific Ags, 30 types of *Staph. aureus* have been identified
 - Coagulase-negative staphylococci do not have type specific Ags

3. Other typing methods

These methods include ribotyping, DNA finger printing and antibiogram typing.

SN ■ Describe the pathogenicity of *Staph. aureus*.

- It is an important pyogenic bacterium
- The lesions produced are localized in nature, in contrast to streptococcal lesions, which are spreading type in nature
- The characteristic feature of *Staph. aureus* is thick creamy pus
- Diseases caused by staphylococci can be divided as:
 1. Superficial and deep infections
 2. Toxin-mediated diseases

Superficial and Deep Infections

1. **Skin infections**—folliculitis, boils, impetigo, carbuncles, styes, pemphigus neonatorum, wounds and burns infections
2. **Respiratory**—tonsillitis, pharyngitis, sinusitis, otitis, bronchopneumonia, lung abscess, empyema

3. **Central nervous system infections**—meningitis, brain abscess, intracranial thrombophlebitis
4. **Endovascular infections**—bacteraemia, septicaemia, pyaemia, endocarditis, pericarditis
5. **Urinary infections**—can cause infection in association with local instrumentation, diabetes, etc.
6. **Musculoskeletal**—osteomyelitis, arthritis, bursitis, pyomyositis

Toxin-mediated Diseases

1. Food poisoning
2. Staphylococcal scalded skin syndrome
3. Toxic shock syndrome

■ Write a short note on toxin-mediated diseases caused by *Staph. aureus*.

Following are the toxin-mediated diseases:

Food Poisoning

- *Staph. aureus* causes a toxin type of food poisoning, characterized by nausea, vomiting and diarrhoea.
- Symptoms appear 2–6 hours after ingestion of food contaminated with preformed toxin
- Type of food responsible for it is meat, fish, milk, milk products, etc. cooked and left at room temperature after contamination with staphylococci for enough time for toxin to accumulate. Source of infection is usually the person who handles food and is a carrier

Staphylococcal Scalded Skin Syndrome

- Staphylococcal scalded skin syndrome (SSSS) is an exfoliative skin disease in which outer layer of epidermis gets separated from the underlying tissue
- Severe form of SSSS is Ritter's disease in newborn and toxic epidermal necrolysis in adults; others are pemphigus neonatorum and bullous impetigo

Toxic Shock Syndrome (TSS)

It is a multi-system illness characterized by fever, headache, congestion, conjunctival redness, subcutaneous oedema, vomiting, diarrhoea, desquamation of hands and feet, progressing to acute renal failure, disseminated intravascular coagulation, hypotensive shock and death.

✓ SN ■ Describe in brief the laboratory diagnosis of *Staph. aureus* infection.

Laboratory diagnosis of *Staph. aureus* infection:

Specimens

Type of specimens collected depends on the type of infection, e.g.

- Pus from pyogenic lesions
- Cerebrospinal fluid in meningitis
- Blood in septicaemia
- Vomitus or faeces or food in food poisoning
- Urine in urinary tract infections
- Sputum in respiratory infections, etc.

Collection

Sample is collected in a sterile container by using standard procedures.

Transport

Transported to laboratory as early as possible.

Processing of Specimen**Microscopic Examination**

- Wet preparation of liquid specimen—shows pus cells
- Gram stain—Gram-positive cocci in clusters along with pus cells

Culture

- Specimens are routinely inoculated on blood agar and when staphylococci are suspected to be scanty or outnumbered by other bacteria, e.g. swabs from carriers or faeces from food poisoning, specimen is also inoculated on selective media such as:
 - Salt milk agar
 - Mannitol salt agar
 - Ludlam's media
- Incubated overnight
- Typical staphylococcal colonies are processed further for identification and confirmation by Gram staining of culture smear and biochemical reactions

Biochemical Reactions

- Catalase test-positive—to differentiate it from streptococci
- Coagulase test-positive in *Staph. aureus*—to differentiate *Staph. aureus* from other species of staphylococci (Fig. 9.11)

Serology

Useful in deep infections only Antistaphylolysin titre—more than 2 units/ml or rising titre is significant.

- **Antibiotics Sensitivity Testing**

It is done by Kirby-Bauer disc diffusion method, antibiotics used are penicillins, cefoxitin, gentamicin, erythromycin, clindamycin, tetracycline, cotrimoxazole, vancomycin, etc.

■ **Write a note on Methicillin resistant *Staph. aureus* (MRSA).**

- MRSA are strains resistant to all beta lactam antibiotics, hence their detection in laboratory is important.

Methods to detect MRSA

1. Susceptibility to cefoxitin disc-resistance to cefoxitin indicates MRSA
2. Chromogenic media
3. MIC determination by agar or broth dilution method/E-test
4. Latex agglutination test
5. PCR
6. DNA probes

Treatment

Vancomycin and linezolid. Nasal topical 2% mupirocin. Daily shower with chlorhexidine based soaps

Prevention and control of MRSA

- Isolation of colonized and infected patients
- Hand hygiene
- Use of alcoholic chlorhexidine solution for hand wash
- Screening of contacts and staff

■ How are *Staph. aureus* infections treated?

- Treatment should always be guided by antibiotic sensitivity testing because of the problem of drug resistance in staphylococci
- Mild topical lesions are treated with bacitracin
- Benzyl penicillin, erythromycin, oxacillin, vancomycin, etc. are commonly used
- Penicillinase producing staphylococci are treated with erythromycin, methicillin and cloxacillin
- Methicillin resistant *Staph. aureus* are treated with vancomycin

■ Mention the characteristic features of *Staph. epidermidis* and *Staph. saprophyticus*.***Staph. epidermidis***

- It is a coagulase-negative staphylococci normally present on the skin
- It is usually nonpathogenic but can cause infection when host defenses are breached
- It is a common cause of stitch abscess
- It can also cause septicaemia because it has predilection to grow on implanted foreign body such as heart valves and shunts
- It also causes endocarditis in drug addicts
- It is sensitive to novobiocin

Staph. saprophyticus

- It is a coagulase-negative staphylococci, may be normally present on skin and periurethral area
- It is pathogenic. It causes urinary tract infections in young females
- It is resistant to novobiocin

30

Chapter

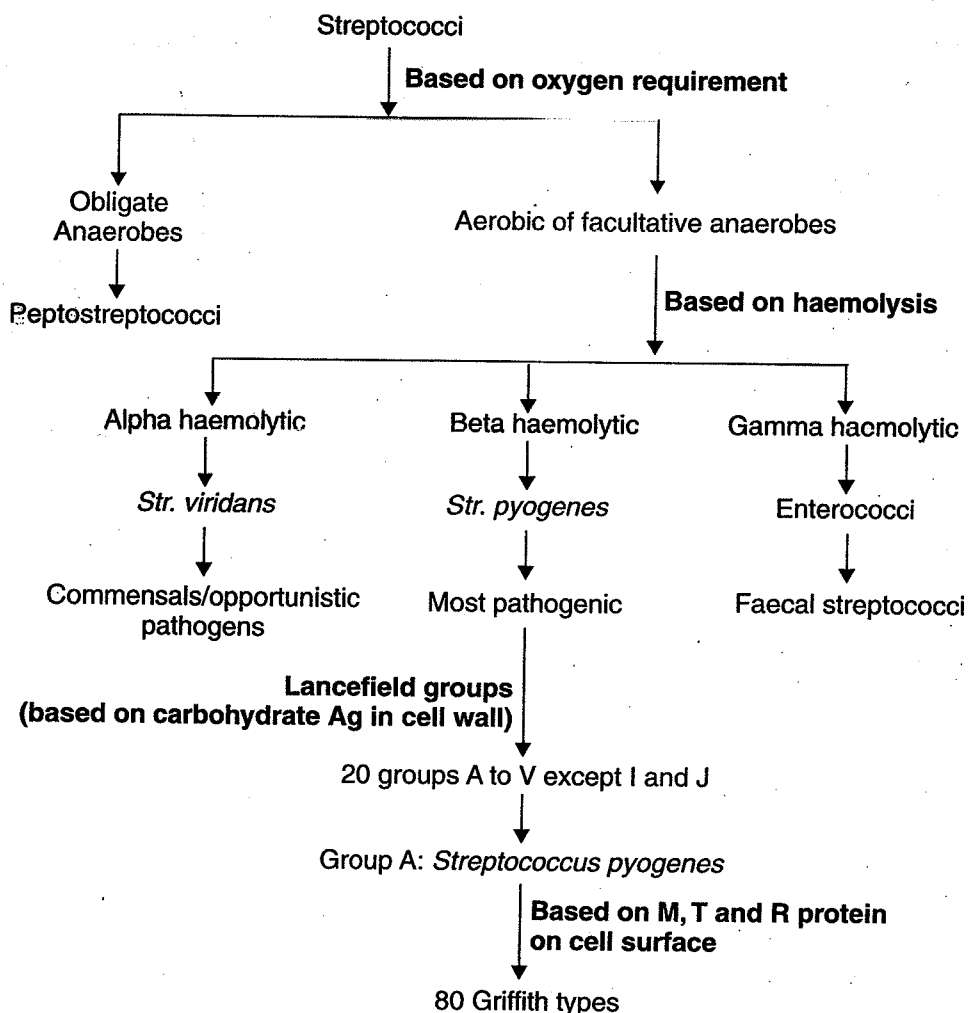
Streptococcus

■ Name the scientists associated with the discovery of streptococci.

Billroth (1874) first seen in erysipelas and wound infections and named them streptococci (streptos means twisted or coiled). Ogston (1881) isolated them from acute abscesses. Rosenbach (1884) isolated the cocci from suppurative lesions in human beings and named them *Streptococcus pyogenes*.

✓ SN ■ Classify streptococci.

Based on oxygen requirement and haemolysis streptococci are classified as follows (Flowchart 30.1).



Flowchart 30.1 Classification of streptococci.

■ Differentiate between alpha-, beta- and gamma-haemolysis.

Differentiating features of alpha-, beta- and gamma-haemolysis are presented in Table 30.1.

Table 30.1 Differences between alpha-, beta- and gamma*-haemolysis

Alpha-haemolysis	Beta-haemolysis
It is greenish in colour	It is a clear zone (colourless)
It is a zone with partial haemolysis (unlysed RBCs are present)	It is a zone of complete haemolysis (all RBCs in the zone are lysed)
Zone of lysis is 1–2 mm in width	Zone of lysis is 2–4 mm in width
Zone has indefinite margin	Zone has sharp defined margin

*Gamma haemolysis means no haemolysis and it does not produce any change in media.

✓ **SN** ■ Enumerate the important morphological features of *Str. pyogenes*.

Morphological Features

- These are Gram-positive cocci arranged in chains
- Size: 0.5–1 μ in diameter
- Chain formation occurs because cocci divide in one plane and the daughter cells fail to separate completely
- Nonmotile and nonsporing
- Some strains have capsule—groups B, C and D
 - Group B and D—polysaccharide capsule
 - Group C—hyaluronic acid capsule (Fig. 30.1)

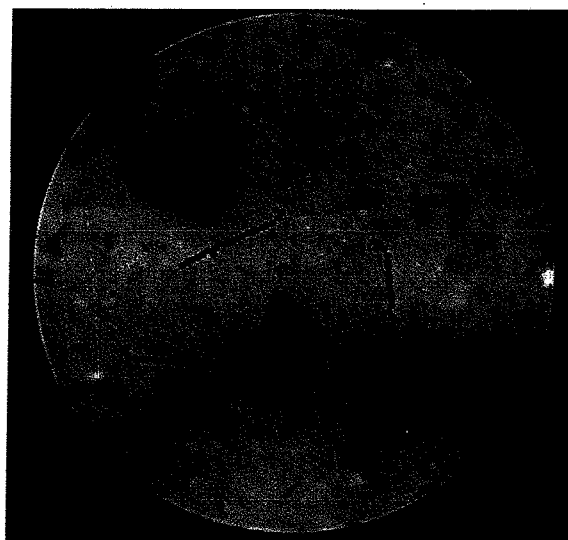


Fig. 30.1 Streptococci.

■ Mention the cultural characteristics of *Str. pyogenes*; include media and colony characters.

Cultural Characters

- Aerobe and facultative anaerobe, grow better in presence of 5–10% CO₂
- Optimum temperature 37°C (range, 22°–40°C)
- Grow on enriched media

Media and Colony Characters

- Media—blood agar, selective media—crystal violet blood agar and blood agar with polymyxin, neomycin sulphate and fusidic acid (PNF media)
- Blood agar—small colonies, 0.5–1 mm, circular, semitransparent, low-convex disc with an area of beta-haemolysis around them (Fig. 30.2)

■ List the biochemical reactions, which are specific to *Str. pyogenes*.

- Ferments sugars with acid
- Catalase—negative. It helps to differentiate it from staphylococcus
- Bile solubility—negative. It helps to differentiate it from pneumococci

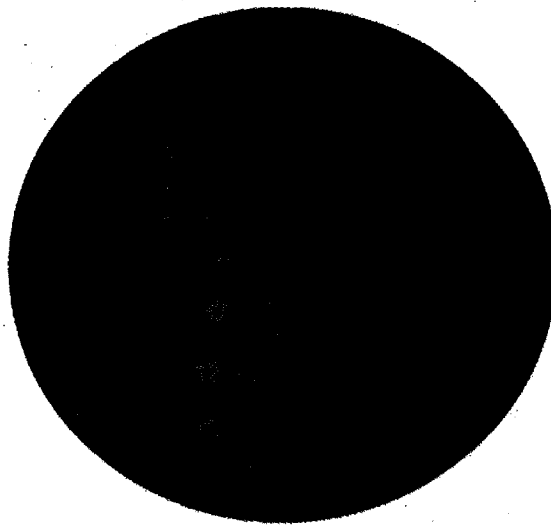
■ **To which factors is *Str. pyogenes* resistant or sensitive?**

- It is a delicate organism that can easily destroyed by heat (54°C for 30 minutes) and antiseptics
- It is resistant to crystal violet, hence used as selective agent in media
- Sensitive to many antibiotics and does not develop resistance
- Sensitivity to bacitracin helps in identification of *Str. pyogenes*

■ **Mention the type of antigens present in *Str. pyogenes*.**

Antigens in *Str. pyogenes* are present in the capsule and cell wall.

Fig. 30.2 *Str. pyogenes* colonies on blood agar.



- **Capsular Ag**—when present inhibits phagocytosis
- **Cell wall Ag**—group-specific carbohydrate and protein Ags
- **Group specific carbohydrate Ag**
 - It forms middle layer of cell wall
 - On the basis of it streptococci are divided into 20 Lancefield groups
 - Typing is done by precipitation in a capillary tube
- **Protein Ags**
 - They are M, T, R present on the surface and form outer layer of cell wall
 - M is more important in virulence and classification
 - They are typed in 80 Griffith types by Griffith typing
 - M typing is done by capillary precipitation
- **Other Ags**—cell surface proteins—protein F and protein G

SN ■ **Write short notes on the virulence factors produced by *Str. pyogenes*.**

Antigens

- **Capsular Ag**—when present inhibits phagocytosis
- **Protein antigen**—inhibits phagocytosis and forms fimbriae, which are covered by lipopolysaccharides (LPS) and help the organism to attach to the cell
- **Carbohydrate C Ag**—plays role in nonsuppurative sequelae of streptococcal infection because of antigenic cross reactions with antigen of heart and kidney
- **Protein F**—may help in attachment to pharyngeal wall
- **Protein G**—attaches to Fc portion of IgG and may hinder Ab-binding and prevent phagocytosis

Toxins

Haemolysins and erythrogenic toxin.

Haemolysins

Two haemolysins—haemolysin O and haemolysin S

- **Properties of haemolysin O (streptolysin O)**
 - It is oxygen labile, hence haemolysis is seen only in pour plates and anaerobic culture
 - It is strongly antigenic and induces ASO Abs, which help in diagnosis of streptococcal infections
 - Also has action on neutrophils, platelets and cardiac tissue

- **Properties of haemolysin S (streptolysin S)**

- It is soluble in serum
- It produces haemolysis around surface of colonies
- It is nonantigenic
- It inhibits chemotaxis and phagocytosis

Erythrogenic toxin

- It is responsible for rash in scarlet fever
- The strains producing it carry a prophage DNA, which code for toxin production
- It is pyrogenic
- It can cause immunosuppression and increase sensitivity of host to streptococcal toxins
- It is used in Dick test, which detects susceptibility to scarlet fever

Enzymes

Some important enzymes that significantly contribute towards spread of infection are:

1. Streptokinase
2. Deoxyribonucleases
3. Hyaluronidase
4. Proteinase
5. NADase

Streptokinase

It has fibrinolytic activity for human fibrin. Hence, it is partially responsible for spread of infection by preventing formation of fibrin at the infected site.

Streptodornase or deoxyribonuclease

- It degrades DNA, which is accumulated in pus as a result of disintegration of neutrophils
- Depolymerization of DNA is responsible for serous character of streptococcal pus
- It contributes towards spread of infection
- It is of four types—A, B, C, D
- Type B DNase is antigenic, induces anti-deoxyribonuclease Abs, these Abs are helpful in diagnosis of skin infection where ASO titre is low

Hyaluronidase

- It splits hyaluronic acid, which binds the tissue cells together
- It facilitates the spread of streptococci.
- It is antigenic and Abs to it are formed after infection

Proteinase

It destroys protein.

NADase

May have leucotoxic effect.

✓ **SN ■ Mention the types of infections caused by streptococci.**

Streptococci cause:

1. Suppurative infections
2. Nonsuppurative complications

Suppurative infections

- **Respiratory tract infections**—Sore throat, tonsillitis, pharyngitis, scarlet fever, pneumonia
- **Skin infections**—Erysipelas, impetigo, lymphangitis, cellulitis, pyoderma, wound infections, burns infection, necrotizing fasciitis, etc.
- **Genital infections**—Puerperal fever and puerperal sepsis
- **Other infections**
 - Otitis media, mastoiditis, peritonsillar abscess
 - Suppurative cervical lymphadenitis
 - Ludwig's angina, meningitis
 - Septicaemia, pyaemia, endocarditis
 - Abscesses in internal organs such as brain, lung, liver and kidney

Nonsuppurative complications

- Acute rheumatic fever
- Acute glomerulonephritis

■ Write a note on suppurative infections.**Suppurative Infections****1. Respiratory Tract Infections**

- Primary site involved is throat, causing sore throat
- It is the commonest streptococcal disease
- It may lead to pharyngitis and tonsillitis
- Streptococcal pneumonia is usually secondary to viral infections of lung
- Scarlet fever is an uncommon disease characterized by sore throat and erythematous rash with punctate spots

2. Skin and Soft Tissue Infections

- It causes pyogenic infections of skin, such as wound and burns infection
- It can cause lymphangitis and cellulitis
- Erysipelas—it is a disease causing spreading sharply demarcated erythematous lesions seen in adults
- Impetigo—it is a skin infection in young children characterized by a superficial skin lesion as discrete spot of 2–5 cm in diameter and heals without scar
- Streptococcal pyoderma is more severe ulcerative skin lesion
- Necrotizing fasciitis is caused by aerobes and anaerobes as a mixed infection or streptococci alone. Clinically, it shows extensive necrosis of subcutaneous tissue, muscle and fascia along with TSS-like systemic involvement in the form of disseminated intravascular coagulation (DIC) and multi-system failure—streptococcal toxic shock syndrome

3. Genital Infections

Str. pyogenes are responsible for puerperal sepsis and fever. The source is exogenous—usually medical and paramedical staff and contaminated instruments.

4. Other Suppurative Infections

- Spread of infection from pharynx or tonsil lead to otitis media, mastoiditis, sinusitis peritonsillar abscess and cellulitis, Ludwig's angina, cervical lymphadenitis
- Septicaemia and pyaemia may occur leading to meningitis, endocarditis and abscesses in internal organs

■ **Write a note on nonsuppurative complications produced by *Str. pyogenes*.**

Nonsuppurative Complications

1. Acute Rheumatic Fever

- *Str. pyogenes* causes acute rheumatic fever involving heart and joints
- It usually develops in small percentage of cases, after 2–3 weeks of pharyngeal infection, which is persistent and repeated
- Mechanism may be
 - Ag cross-reaction between Group A streptococci and myocardium. So Abs produced against streptococcus A cross react with heart causing damage
 - Direct toxic effect may occur
 - Lesions may be due to hypersensitivity and autoimmunity

2. Acute Glomerulonephritis

- It is due to cross reaction between Abs produced against streptococci and Ag of glomerular basement membrane or deposition of Ag–Ab complexes and complement activation
- It usually follows infection with nephritogenic strains of streptococci

✓ **State the procedure followed and examinations performed in the laboratory diagnosis of *Str. pyogenes* infection.**

In suppurative infections diagnosis is made by culture while in nonsuppurative complications it is made by antibody detection.

Specimens are collected by appropriate procedures in sterile containers:

- Blood in septicaemia and in nonsuppurative complications
- Pus in pyogenic infections
- CSF in meningitis
- Throat swab and sputum in respiratory infections
- Vaginal swab—in genital infections
- Other samples based on site of infection

Transport

As it is a delicate bacterium, swab should be transported in Pike's media.

Microscopic Examination

- It is important in pus and cerebrospinal fluid
- Smears of throat swab and vaginal swab for demonstration of bacteria are not of diagnostic value
 - **CSF wet preparation**—plenty of pus cells
 - **Gram stained smear**—Gram-positive cocci in chains along with pus cells
 - **Fluorescent antibody technique**—it is for detection of bacteria in smears by using fluorescent tagged antibody and has more sensitivity than Gram's stain

Culture

- Blood agar is used usually and selective media are used for specimens from vagina, throat, etc. where commensals are present
- **Selective media**—crystal violet blood agar and PNF media
- Growth is promoted by 5–10% CO₂ supplementation
- Typical colonies with beta-haemolysis on blood agar are processed for further identification by biochemical reactions

Biochemical Reactions

- Catalase—negative
- Bile solubility—negative

Bacitracin Sensitivity

- Bacitracin disc with 0.04 mg of bacitracin placed on inoculated culture plate of streptococci
- After 24 hours observed for zone of inhibition
- Wider zone of inhibition is observed only with *Strep. pyogenes*
- Bacitracin sensitivity is helpful for identification of *Strep. pyogenes*

Antibiotic Sensitivity Testing (AST)

Isolate is tested by Kirby–Bauer disc diffusion method on Mueller–Hinton agar with 5% blood using penicillin, erythromycin, cephalosporins, tetracycline, fluoroquinolones, etc.

Ag Detection

ELISA and agglutination test.

Nucleic Acid Detection

DNA probes.

Ab Detection

1. Streptozyme test—It is passive haemagglutination test using RBCs coated with antigen
2. Antistreptolysin O test (ASO test)—It is a slide agglutination test, can be done qualitatively and quantitatively. Significant titre of 1:200 or more indicates prior streptococcal infection
3. Anti-deoxyribonuclease B test—Significant titre 1:300

■ **What treatment and prophylaxis measures should be taken against *Str. pyogenes*?**

Treatment

Administration of Penicillin G, erythromycin, cephalosporins, etc.

Prophylaxis

For prevention of rheumatic fever, the long-term administration of penicillin in children, who have developed early signs of rheumatic fever.

■ **Describe in brief Group B streptococci and Group D streptococci.**

Group B Streptococci**Species of Medical Importance**

Str. agalactiae.

Pathogenicity

These are major pathogens in neonates and young children. Infection in neonate may be early or late onset.

Early onset	Late onset
1. Occurs within a week of birth	Develops after 2nd week
2. Present as septicaemia, meningitis and it is fatal	Not fatal but leaves residual effect
3. Infection acquired from maternal vagina during birth	Infection is exogenous, acquired from hospital environment

They can also cause infection in adults as local genital septic lesions and puerperal sepsis.

Identification

- Hippurate hydrolysis—Positive
- CAMP reaction (Christie–Atkins and Munch–Paterson)—Positive

Group D Streptococci**Species of Medical Importance**

Includes enterococci and non-enterococci:

- Commonest: *Enterococcus faecalis*
- Others: *E. faecium*, *E. durans*, *E. avium*

Pathogenicity

- Urinary tract infections
- Wound infections
- Septicaemia
- Peritonitis
- Biliary tract infections

Identification

- Ability to grow in presence of 40% bile
- Ability to hydrolyze aesculin

■ Write a note on alpha-haemolytic streptococci (*Str. viridans*).

- Alpha-haemolytic streptococci are present as commensal in mouth and pharynx
- Produce alpha-haemolysis on blood agar and do not grow on MacConkey's agar

Medically Important Species

- *Str. salivarius*
- *Str. mutans*
- *Str. sanguis*
- *Str. mitior*
- *Str. milleri*

Pathogenicity

- Dental caries
- Endocarditis in patients with pre-existing cardiac lesions

Dental Caries

- *Str. mutans* is commonly involved
- It breaks down dietary sucrose to acid and dextran, acid damages dentine and dextran binds with food debris, mucus, epithelial cells, bacteria to form plaques leading to dental caries

Endocarditis

Str. sanguis and *Str. mitior* are commonly involved, during tooth extraction or other dental procedures, they cause transient bacteraemia and cause infective endocarditis.

Diagnosis of infective endocarditis

It is made by repeated blood cultures.

Treatment

Should be guided by antibiotic sensitivity test, some strains may *develop* resistance to penicillin.

31

Chapter

Pneumococcus

■ Name the scientists associated with the discovery of pneumococci.

- Pasteur and Sternberg independently noticed pneumococci in 1881
- Fraenkel and Weichselbaum independently proved the relationship between pneumococci and pneumonia in 1886

■ State the morphological features of pneumococci.

Morphological Features

- Gram-positive capsulated diplococci (Fig. 31.1a)
- Size: 1 μ in diameter
- Arranged in pairs with broad ends in opposition
- Flame-shaped, elongated with one end broad and the other pointed
- Capsulated—capsule can be demonstrated by:
 - Negative stain (Nigrosin or India ink)—capsule is seen as a clear halo around bacteria (Fig. 31.1b)
 - *Quellung* reaction—capsule can be seen as refractile halo after mixing with homologous type specific antibody, which combines with capsular polysaccharide
- Nonmotile and nonsporing

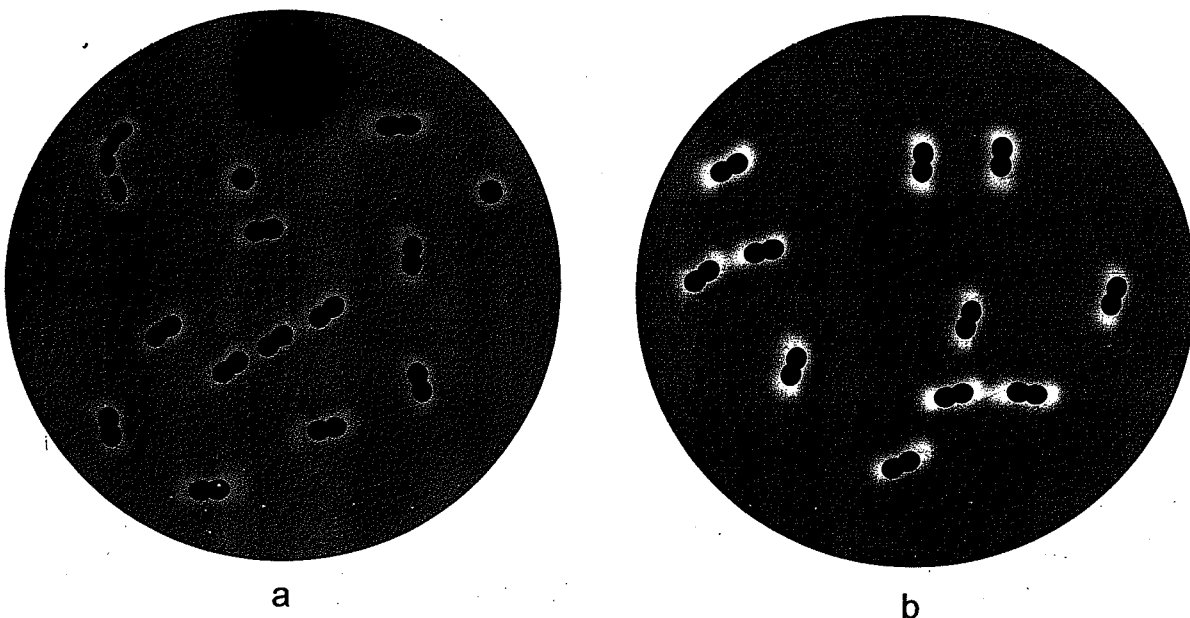


Fig. 31.1 Pneumococci: a. Gram stain, b. Negative stain.

- **Mention the cultural characteristics of pneumococci, include media and colony characters.**

Cultural Characters

- Aerobe and facultative anaerobe
- Growth improved by 5–10% CO₂
- Optimum temperature 37°C (range, 25°–42°C) and optimum pH 7.8
- Grows well on enriched media like blood agar, chocolate agar, etc.

Media and Colony

Blood agar

Small, mucoid, transparent colony with alpha-haemolytic zone. Further incubation leads to central umbonation with raised edges; concentric rings are seen when viewed from top giving draughtsman or carom-coin appearance to colony. Colony under anaerobic incubation is surrounded by beta-haemolysis due to oxygen labile pneumolysin.

- **List the biochemical reactions of pneumococci, along with their results.**

Biochemical Reactions

- Ferments many sugars but only inulin fermentation is helpful in identification
- Fermentation is tested on Hiss's serum slope or serum water
- Bile solubility—they are bile soluble (sodium taurocholate when added on colony causes lysis of pneumococci: colony disappears leaving an area of alpha-haemolysis)
- Catalase—negative
- Oxidase—negative

- **To which factors are pneumococci resistant or sensitive?**

- Pneumococci are delicate, can be killed readily by heat and disinfectants
- Strains resistant to beta-lactam antibiotics and erythromycin have been reported
- They are sensitive to optochin, this test is used for identification purpose—5 µg of optochin disc forms 14 mm zone of inhibition

- **Describe the antigens present in pneumococci.**

Antigens present in pneumococci are described below:

Capsular Ag

- It is a polysaccharide Ag
- The capsular polysaccharide diffuses into the culture, hence called “specific soluble substance”
- It is a virulence factor, and plays role in protecting organism from phagocytosis
- On the basis of capsular Ag, pneumococci are divided into 85 serotypes. Typing is done by
 - *Quellung* reaction: When sample is mixed with equal quantity of type-specific antisera and methylene blue, sharply delineated, refractile capsule is seen
 - Agglutination reaction and precipitation reaction

Somatic Ag

It is a cell wall Ag—also called pneumococcal C substance.

C-reactive Protein (CRP)

- It is an abnormal protein that precipitates C carbohydrate of cell wall
- It appears in acute phase sera of cases of pneumonia but disappears during convalescence
- It also occurs in some other pathological conditions

- It is not an antibody formed in response to pneumococcal infection
- It is an acute phase protein produced in hepatocytes and its production is stimulated by bacterial inflammation, malignancy and tissue destruction
- It is used as an index of treatment in rheumatic fever and in some other diseases. Tested by:
 - Capillary precipitation and latex agglutination test

M Protein

Also contains somatic M protein.

■ Describe in brief virulence factors of pneumococci.

- Polysaccharide capsule inhibits phagocytosis
- IgA protease acts on IgA Abs, which play important role in local immunity
- Protein adhesin—mediates adhesion
- Cell constituents such as
 - Teichoic acid and peptidoglycan—activate alternate complement pathway
 - Pneumolysin—it is cytotoxic and activates classical complement pathway

■ Describe in brief the genetic variation in pneumococci.

- Capsulated pneumococci (smooth forms) are virulent but noncapsulated (rough forms) are avirulent
- Repeated subculture of pneumococci may lead to smooth (S) to rough (R) variation
- In R form, organisms are autoagglutinable, avirulent, noncapsulated and form rough colonies
- Rough pneumococci derived from capsulated cells of one serotype may be made to produce capsule of the same or different serotypes. This is possible by treatment with DNA from respective serotypes of pneumococci
- This transformation may be demonstrated *in vivo* or *in vitro*

■ What diseases are caused by pneumococci? Discuss them briefly.

Diseases caused by pneumococci are:

- **Respiratory infections**
 - Lobar or bronchopneumonia
 - Acute exacerbation of chronic bronchitis
 - Acute tracheobronchitis
 - Empyema
- **Meningitis**
- **Bacteraemia**
- **Others** such as suppurative complications of bacteraemia—conjunctivitis, mastoiditis, suppurative arthritis, pericarditis, otitis media, sinusitis, etc.

Respiratory Infections

- Pneumococci are the commonest cause of pneumonia
- Usually, it is secondary infection (rarely primary)
- It can be lobar pneumonia or bronchopneumonia. Young children and older adults, over 50, usually show—bronchopneumonia and 10–25 years age group show—lobar pneumonia
- It can cause acute exacerbation of chronic bronchitis due to presence of copious secretions, which facilitate pneumococcal invasion

Bacteraemia

It is common during early stage of lobar pneumonia due to diffusion of capsular polysaccharide into the blood and tissue.

Meningitis

It affects all age groups especially the children and the elderly. Spread occurs through nasopharynx to meninges via blood stream or by contiguity. Generally, it is secondary to other pneumococcal infections such as pneumonia.

Others

All other infections usually follow bacteraemia, mostly as complications of pneumonia.

■ Describe in brief the laboratory diagnosis of pneumococcal infections.

Specimens

Sputum, blood, CSF, aspirate of middle ear and laryngeal swabs.

Collection

- Sputum—in leak-proof, wide-mouthed sterile container
- Blood—by venepuncture into glucose broth or taurocholate broth
- CSF—by lumbar puncture in a sterile container
- Aspirate—by syringe

Transport

All samples should be immediately transported to laboratory. Blood should be transported in glucose broth or taurocholate broth

Microscopic Examination

- **Gram stain**—Gram-positive capsulated diplococci along with pus cells
- **Quellung reaction**—to demonstrate capsule

Culture

- Specimen is inoculated on blood agar and chocolate agar with 5–10% CO₂ supplementation
- On blood agar observed for small, moist, mucoid, transparent with alpha-haemolytic colony. Further incubation gives typical draughtsman or carom coin appearance to colony
- These typical pneumococcal colonies are processed further for identification and differentiation from *Str. viridans* (Table 31.1)

Antibiotic Sensitivity Testing

By Kirby–Bauer disc diffusion method on Mueller–Hinton agar with 5% sheep blood using penicillin, cephalosporins, erythromycin, clindamycin, vancomycin, chloramphenicol and sulphonamides.

Ag Detection

In serum and CSF by:

- Latex agglutination test
- Counterimmunoelectrophoresis
- Enzyme-linked immunosorbent assay test
- Coagglutination test

Ab Detection

- Agglutination
- Indirect haemagglutination
- Indirect fluorescent antibody technique
- RIA

Table 31.1 Differences between *Str. pneumoniae* and *Str. viridans*

	<i>Str. pneumoniae</i>	<i>Str. viridans</i>
1. Morphology		
Shape	Lanceolate or flame-shaped cocci	Round-oval cocci
Arrangement	In pairs	In chains
Capsule	Capsulated	Noncapsulated
Quellung reaction	Positive	Negative
2. Cultural characters		
On solid media	Dome shaped, transparent alpha-haemolytic, later gives draughtsman appearance	Alpha-haemolytic colonies
In liquid media	Uniform turbidity	Granular turbidity
3. Tests for identification		
Rile solubility	Positive	Negative
Inulin fermentation	Positive	Negative
Optochin sensitivity	Positive	Negative
4. Animal pathogenicity		
Intraperitoneal inoculation in mice	Produces fatal infection	Nonpathogenic

Animal Inoculation

It is used for samples containing scanty bacteria.

Mouse pathogenicity—It produces fatal infection in mouse following intraperitoneal inoculation, death occurs in 1–3 days and peritoneal exudates and heart blood shows pneumococci.

■ Which antibiotics are used in the treatment of pneumococcal infections?

- Antibiotics used in the treatment of pneumococcal infections are: penicillin, amoxycillin, erythromycin, tetracycline, cephalosporins, vancomycin, fluoroquinolones, etc.
- Penicillin-resistant strains are known to occur and to develop multi-drug resistance.

32

Chapter

Neisseria

■ Name the scientists associated with the discovery of *Neisseria* spp.

- Neisser (1879) was first to describe gonococci in gonorrhoea pus
- Bumm (1885) was first to cultivate the gonococcus and prove its pathogenicity by inoculating human volunteers
- Weichselbaum (1886) was first to describe meningococci from the spinal fluid of a patient

■ Enumerate the distinguishing morphological features of *N. meningitidis*.

Morphological Features

- Gram-negative cocci
- Size: 0.6–1 μ in diameter
- Arranged in pairs with adjacent sides flattened
- Long axis of coccus is at right angle to line joining two cocci
- They are generally intracellular
- Nonmotile and nonsporing
- Noncapsulated (fresh isolates are capsulated)

■ Mention the cultural characteristics of *N. meningitidis*; include media and colony characters as well.

Cultural Characters

- They have exacting growth requirement and cannot grow on ordinary media
- Grow well on enriched media
- They are strict aerobes
- Growth is enhanced with supplement of 5–10% CO₂
- Optimum temperature 35°–36°C and optimum pH 7.0–7.4

Media Used

- Blood agar, chocolate agar, Muller–Hinton starch casein hydrolysate agar
- **Selective media**—modified Thayer–Martin medium (chocolate agar + vancomycin, colistin, nystatin)—useful for contaminated specimens

Colony Characters

- On blood agar, Muller–Hinton starch casein hydrolysate agar—colonies are small, 1 mm in diameter, round, convex, gray, translucent, smooth and glistening with entire margins
- On chocolate agar—colonies are slightly larger than that of blood agar

■ Name the biochemical reactions considered for detecting *N. meningitidis*.

Biochemical Reactions

- Catalase—positive
- Oxidase—positive

- Utilize glucose and maltose by oxidative attack
- Do not attack lactose and sucrose

■ **Name the antigen present in *N. meningitidis*. Classify meningococci on this basis.**

- Capsular antigen—polysaccharide in nature
- On the basis of capsular antigen, meningococci have been divided into 13 serogroups: A, B, C, D, X, Y, Z, 29E, W-135, H, I, K, L
- A, B, C, 29E, W-135 and Y are responsible for large majority of infections

■ **To which external factors is *N. meningitidis* susceptible?**

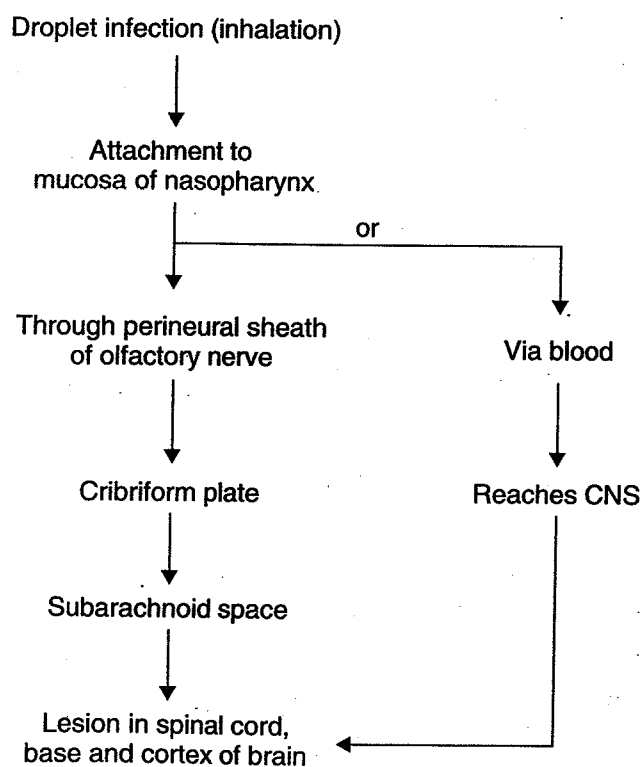
N. meningitidis is a delicate organism, which is susceptible to heat and disinfectants.

■ **Describe in brief the virulence factors of *N. meningitidis*.**

- **Capsular polysaccharide**—acts by inhibiting phagocytosis
- **Pili**—help in adhesion to mucosa
- **Endotoxin**—released during multiplication. Its invasion in cells of vascular endothelium induces inflammatory response and causes vascular necrosis. It is involved in causing petechial haemorrhages in **Waterhouse-Friderichsen syndrome**
- **IgA protease**—excreted in extracellular environment; it cleaves and inactivates IgA—an immunoglobulin involved in mucosal defense
- **Outer membrane proteins**—some outer membrane proteins are involved in attachment to the host cell

■ **Give an outline of the path of disease development in *N. meningitidis* infections.**

The course of disease development in *N. meningitidis* infections is presented in Flowchart 32.1.



Flowchart 32.1 Path of disease development in case of infection by *Neisseria meningitidis*.

■ **Name the diseases caused by *N. meningitidis*. Give their clinical features.**

Pathogenicity

- Meningococcaemia
- Meningitis
- Others—Pneumonia, conjunctivitis, arthritis

Clinical Features

It may cause self-limiting febrile illness and local inflammation with rhinitis and pharyngitis.

Meningococcaemia

- Bacteria enter blood stream from posterior nasopharynx, causing meningococcaemia characterized by fever, malaise, and petechial skin lesions. It may cause lesions in lungs, joints, adrenals and other internal organs due to endotoxin
- A fulminant meningococcaemia called **Waterhouse-Friderichsen** syndrome—characterized by shock, disseminated intravascular coagulation; and multisystem failure may develop. Meningococcaemia is favoured by complement deficiency (factor C5–C9)

Meningitis

Infection of meninges is characterized by headache, neck-stiffness, and vomiting, accompanied by delirium and confusion.

■ **Describe the laboratory diagnosis of *N. meningitidis* infection.**

Specimens

Cerebrospinal fluid, blood, aspirates from skin lesions and joint, nasopharyngeal swab, sputum, and conjunctival swab.

Collection

Samples are collected in sterile containers by using standard procedures.

Transport

- As it is a delicate organism, sample should reach the laboratory immediately
- Swab should be transported in Stuart's transport medium
- Blood should be added to glucose broth and transported

Processing of Specimen

Cerebrospinal fluid looks turbid. It is divided into three portions for further examination:

- 1st for—microscopic examination
- 2nd for—culture
- 3rd for—enrichment

Microscopic Examination

- **Wet preparation**—shows pus cells
- **Gram stain**—shows Gram-negative diplococci and pus cells
- **Direct immunofluorescence test**—smear prepared and treated with antibody conjugated to fluorescent tag shows presence of meningococci

Culture

- Portion of cerebrospinal fluid is inoculated on blood agar and chocolate agar
- Incubated at 35°C with 5–10% CO₂ supplementation

- Observed for small, 1 mm in diameter, round, convex, gray, translucent, smooth, glistening colonies with entire margins
- Blood culture—Positive in early stage of meningitis. Blood is added to glucose broth or trypticase soy broth and subcultured daily for 4–7 days
- Typical colonies are identified by using biochemical reactions

Biochemical Reactions

- Catalase—Positive
- Oxidase—Positive
- Glucose, Maltose—Utilized oxidatively

Third portion of CSF is added to glucose broth for enrichment and culture, repeated if direct plating fails.

Serotyping

Serotyping is done by agglutination with poly or monovalent anti-meningococcal sera.

Antibiotic Susceptibility Testing

It is done by Kirby–Bauer disc diffusion method on Müller–Hinton agar with 5% sheep blood using chloramphenicol, cotrimoxazole, erythromycin, ciprofloxacin, doxycycline, ceftriaxone and ceftazidime. Minimum inhibitory concentration (MIC) values should be calculated to find out susceptibility to penicillin.

Ag Detection

Performed in supernatant of cerebrospinal fluid by using

- ELISA
- Counter immunoelectrophoresis
- Latex agglutination test

Ab Detection

Haemagglutination test is performed to detect Ab in chronic cases.

Nucleic Acid Detection in Samples

By polymerase chain reaction.

- Which antibiotics are administered in the treatment of *N. meningitidis* infections? Suggest prophylaxis for such infections.

Treatment

Antibiotics used in the treatment of *N. meningitidis* infections are penicillin, chloramphenicol, erythromycin, ciprofloxacin, doxycycline, ceftriaxone and ceftazidime.

Prophylaxis

Monovalent and polyvalent vaccines containing capsular polysaccharides of groups A, C, W-135 are available. Immunity is group specific.

- State morphological features of *N. gonorrhoeae*.

- Gram-negative cocci
- Size: 0.6–1 μ in diameter
- Pear- or bean-shaped and possess pili on surface

- Arranged in pairs with adjacent sides concave
- Nonmotile and nonsporing
- Capsulated (Fig. 32.1).

■ **State the cultural characteristics of *N. gonorrhoeae*; include media and colony characters.**

Cultural Characters

- They have exacting growth requirement and cannot grow on ordinary media
- Grow well on enriched media
- Aerobe but can grow anaerobically
- Growth needs supplement of 5–10% CO₂
- Optimum temperature 35°–36°C and optimum pH 7.2–7.4

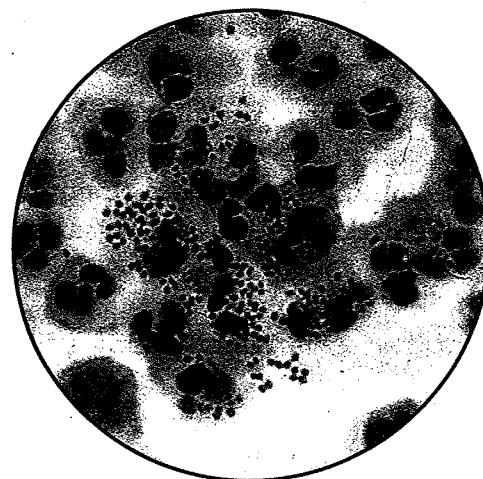


Fig. 32.1 *Neisseria gonorrhoeae*. (Source: Kumar & Clark's Clinical Medicine, Figure 4.37, Saunders Ltd., 2012.)

Media

Blood agar, chocolate agar, and Thayer–Martin medium (containing colistin, nystatin, and vancomycin) and modified Thayer–Martin medium (containing additional trimethoprim).

Colony

- Small, round, translucent, gray, convex or slightly umbonate, finely granular
- On the basis of colony appearance, autoagglutinability and virulence, Kellogg divided colonies into four types: T₁, T₂, T₃ and T₄
 - **Type T₁ and T₂**—small, brown colonies produced by piliated-strains, autoagglutinable and virulent gonococci
 - **Type T₃ and T₄**—are produced by nonpiliated avirulent strains which form smooth suspension
- Fresh isolates produce type T₁, type T₂ colonies and, on subculture they become T₃ or T₄ type of colonies

■ **Which biochemical reactions are specific for *N. gonorrhoeae*?**

Biochemical reactions:

- Catalase—positive
- Oxidase—positive
- Utilize glucose only with production of acid

■ **Mention the antigens present in *N. gonorrhoeae*.**

N. gonorrhoeae have:

- Pili—protein
- Outer membrane proteins—I, II, III
- IgA protease
- Lipopolysaccharide
- Capsule

■ **Mention 'resistance' in *N. gonorrhoeae*.**

- *N. gonorrhoeae* is a very delicate organism
- Heat drying and antiseptics readily kill it
- Freeze drying is a method for long-term storage

■ **Describe in brief the virulence factors in *N. gonorrhoeae*.**

Virulence factors are as follows:

Capsule

It is present in freshly isolated strains. It is polyphosphate, does not prevent phagocytosis but allows intracellular survival of organisms.

Pili

Help in attachment by recognizing specific receptors on host cells and also inhibit phagocytosis.

Outer Membrane Proteins

- **Protein I**—also called *por* protein and **protein III** also called *Rmp* protein. Both act as ligand, attaching the coccus to host cells. Protein I forms pores and protein III is associated with it
- **Protein II**—*Opa* protein—appears to be associated with adherence of gonococci to host cells and also for clumping of cocci in urethral exudates. It has antiphagocytic role

Lipopolysaccharide

Toxicity of infection is due to endotoxic effect of lipopolysaccharide.

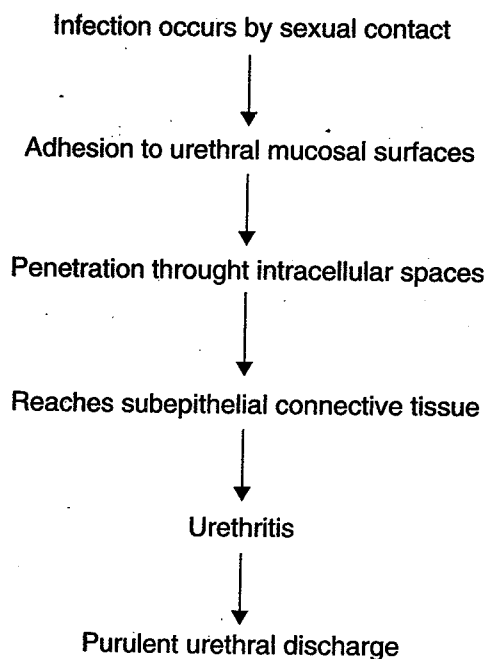
IgA Protease

It cleaves IgA and inactivates it.

■ **Outline a sketch of the path of disease development in *N. gonorrhoeae* infection. State the clinical features.**

Pathogenesis (Flowchart 32.2)

N. gonorrhoeae is the cause of the sexually transmitted disease—Gonorrhoea.



Flowchart 32.2 Course of disease development in *N. gonorrhoeae* infection.

Clinical Features

In Males

- Disease starts as **acute urethritis** with mucopurulent discharge through urethra
- Infection may extend along the urethra to the prostate, seminal vesicles and epididymis
- It may lead to stricture formation in chronic cases
- Can cause **conjunctivitis** due autoinoculation by fingers
- It can cause **proctitis** as a result of anal sex
- Blood invasion and metastatic lesions may occur
- It can also cause **water-can perineum**, characterized by spread of infection to periurethral tissue causing abscesses and multiple discharging sinuses

In Females

- **Cervicitis**—infection involves urethra and cervix-uteri
- Infection may extend to Bartholin's glands, endometrium and Fallopian tube causing **pelvic inflammatory disease, salpingitis**, leading to sterility
- Asymptomatic carriage in cervix is also common
- It can cause **proctitis** by direct contiguous spread in women
- Can cause **conjunctivitis** due to autoinoculation by finger
- Blood invasion and metastatic lesions such as **arthritis** may occur

In Prepubertal Girls

It can cause **vaginitis** in prepubertal girls. **Vulvovaginitis** is not common in adult females because of the stratified squamous epithelium and acidic pH of vaginal secretion.

In Newborns

A nonvenereal infection is **gonococcal ophthalmia** in the newborn. It is a result of direct infection from birth canal.

■ Describe the laboratory diagnosis of *N. gonorrhoeae* infection.

Specimens

- In males
 - Purulent urethral discharge
 - Morning drop of secretion
 - Exudates obtained by prostatic massage
 - Urine
 - In asymptomatic patients—exudates adherent to mucosa
- In females
 - Cervical swab
 - Exudates from urethra
 - Vaginal swab
- In both sexes
 - Anal swab
 - Blood
 - Conjunctival swab

Collection

- **Purulent urethral discharge:** It may be expressed at anterior urethra and collected with swab. Dacron or rayon swabs are preferred instead of cotton swab (may contain fatty acid that inhibit gonococci). Alternatively, it can be collected directly with a loop

- **Exudates adherent to urethra:** Swab is inserted about 2–3 cm into anterior urethra and rotated gently
- **Urine:** Patient is asked to collect fresh midstream urine sample in a sterile container
- **Cervical swab:** Sterile swab is inserted into endocervical canal, moved gently and left in place for 20–30 seconds for absorption of bacteria on swab
- **Urethral exudates:** It is collected by loop or swab
- **Vaginal swab:** It is collected from posterior vault or vaginal orifice
- **Anal swab:** It is collected by inserting swab in anal canal about 4–5 cm and moved, left for 20–30 seconds to collect material from crypts
- **Other specimens:** These collected by using standard procedures (refer to Chapter 28)

Transport

- As gonococci are delicate, sample should reach the laboratory immediately after collection, bedside inoculation is preferred
- All swabs are transported to laboratory in Amies transport media
- Blood and synovial fluids are collected in trypticase soy broth

Microscopic Examination

- **Gram stain**—Shows plenty of pus cells and Gram-negative diplococci, majority are intracellular
- **Fluorescent antibody technique**—It has increased sensitivity and specificity. Smear is prepared and treated with antibody conjugated to fluorescent tag - shows presence of bacteria

Culture

- Swab is directly plated on culture media
- Blood agar, chocolate agar and selective media are used
- All genital-rectal samples should be inoculated on selective media also
- Small, round, translucent, gray, convex or slightly umbonate, finely granular colonies are processed further for identification by using biochemical reactions

Biochemical Reactions

- Catalase—Positive
- Oxidase—Positive
- Fermentation of glucose occurs with acid production
- Does not ferment maltose—Negative

Antibiotic Susceptibility Testing

By Kirby–Bauer disc diffusion method on gonococcus agar with growth supplements using ceftriaxone, ciprofloxacin, ofloxacin, tetracycline, doxycycline, erythromycin, etc. Gonococci are known to develop resistance to penicillin.

Antibody Detection

Useful in patients with metastatic lesions especially when isolation fails. The tests available are:

- Radioimmunoassay
- ELISA
- Haemagglutination

■ Which antibiotics are used in the treatment of *N. gonorrhoeae* infections?

- Antibiotics used in the treatment of *N. gonorrhoeae* infections are: penicillin, erythromycin, ciprofloxacin, doxycycline, and ceftriaxone.

33

Chapter

Corynebacterium

■ Name the scientists associated with the discovery of *Corynebacterium diphtheriae*.

- Bretonneau (1826) was first to recognize the disease and named it diphtheria
- *Corynebacterium* was first observed by Klebs (1883) and first cultivated by Loeffler (1884) and hence called Klebs–Loeffler bacillus (KLB)
- Roux and Yersin (1888) discovered the diphtheria exotoxin. Behring (1890) described the antitoxin

■ Mention the species of *Corynebacterium*.

- *C. diphtheriae*
- *C. ulcerans*
- *C. pseudotuberculosis*
- *C. jeikeium*
- *C. diphtheriae* is an important human pathogen.

■ Enumerate the characteristic morphological features of *C. diphtheriae*.

Morphological Features

- Thin, slender Gram-positive bacilli
- About $3-6 \mu \times 0.6-0.8 \mu$
- They are pleomorphic, show granular or uneven staining
- **Arranged in Chinese letter pattern** (at various angles, resembling letter V, L) **cuneiform pattern** due to incomplete separation of the daughter cells after binary fission
- Nonmotile, noncapsulated and nonsporing
- They may appear club-shaped because of presence of **metachromatic granules** at the ends (Fig. 33.1)
 - Granules consist of polymetaphosphate
 - They are called metachromatic granules as they take bluish purple colour when stained with methylene blue. They are also known as volutin granules or Babes–Ernst granules
 - They are always situated at pole of bacilli, therefore also called polar bodies
 - Special stains for granules are—Albert's stain, Neisser's stain, Ponder's stain
 - They represent energy storage
- Presence of granules in slender Gram-positive bacilli helps in distinguishing diphtheria bacilli from nonpathogenic corynebacteria, which lack them

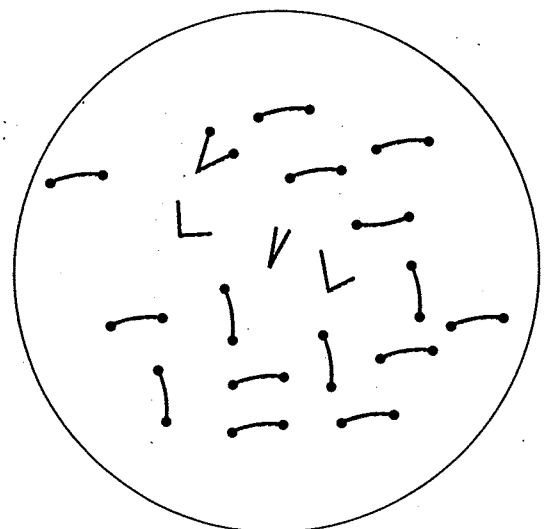


Fig. 33.1 *Corynebacterium diphtheriae*.

■ **State the cultural characteristics of *C. diphtheriae*; include media and colony characters.**

Cultural Characters

- Aerobes and facultative anaerobes
- Optimum temperature 37°C (range, 15°–40°C) and optimum pH 7.2
- Growth improved on enriched media

Media and Colony Characters

- **Loeffler's serum slope:** *C. diphtheriae* grows rapidly in 6–8 hours on this media. It produces colonies, which are small, white, circular, and glistening with irregular edges
- **Blood potassium tellurite (BPT):** It is a selective medium; potassium tellurite is a selective component. *C. diphtheriae* grows slowly on this media producing gray to black colony due to reduction of potassium tellurite to potassium tellurium (Fig. 33.2)

■ **Name the three biotypes of *C. diphtheriae* distinguished by McLeod.**

McLeod classified *C. diphtheriae* into three biotypes based on colony characters on BPT (Table 33.1). These three biotypes are:

1. *Gravis*
2. *Intermedius*
3. *Mitis*

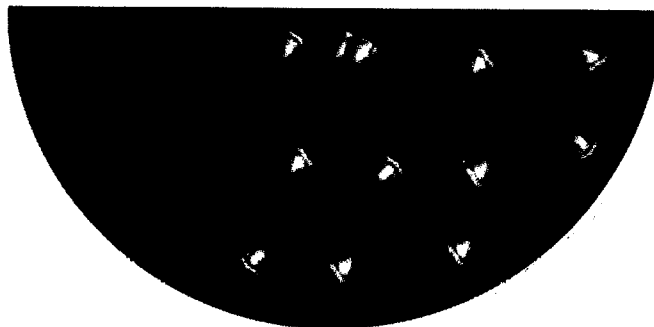


Fig. 33.2 Growth of *C. diphtheriae* on BPT medium.

Table 33.1 Differences between biotypes of *C. diphtheriae*

	<i>Gravis</i>	<i>Intermedius</i>	<i>Mitis</i>
1. Morphology			
a. Length	Short rods	Long forms	Long curved forms
b. Staining	Uniform staining	Irregular staining	Pleomorphic
c. Granules	Few granules	Poor granules	Prominent granules
2. Culture			
a. Blood agar	Variable	Nonhaemolytic	Haemolytic
b. BPT	1–2 mm Gray, raised centre, crenated edge Daisy head colony	1 mm Gray-black, dull granular centre with glistening periphery and lighter ring near edge Frog's egg colony	Variable Shiny-black, circular, convex, smooth Poached egg colony
c. Consistency of colony	Brittle, not easily Emulsifiable	Intermediate between gravis and mitis	Soft buttery, easily emulsifiable
3. Biochemical reactions			
a. Glycogen fermentation	positive	negative	negative
b. Starch fermentation	positive	negative	negative
4. Virulence	Severe	Moderate	Mild
5. Usual complication	Paralytic	Haemorrhagic	Haemorrhagic, obstructive

■ **Mention the distinctive biochemical reactions of *C. diphtheriae*.**

Distinctive biochemical reactions of *C. diphtheriae* are as follows:

- Fermentation of sugar in Hiss's serum media with production of acid only, pathogenic diphtheria ferment sucrose and mannitol
- Starch and glycogen fermentation—*gravis* ferments both, *intermedius* and *mitis* ferment neither
- Nitrate reduction—positive
- H₂S—positive

■ **Mention the resistance of *C. diphtheriae*.**

- It is readily destroyed by heat 58°C for 10 minutes and by disinfectants
- Remains virulent in blankets and dust for 5 weeks

■ **Which antigens are produced by *C. diphtheriae*?**

- Protein antigen on surface (K Ag)
- Polysaccharide O Ag

On the basis of agglutination reaction *gravis*, *intermedius* and *mitis* are classified into 13, 4 and 40 serotypes respectively.

■ **Which factor in *C. diphtheriae* is responsible for virulence? Give its mechanism of action and mode of synthesis.**

- Toxin in *C. diphtheriae* is responsible for virulence
- It is an exotoxin. Strain universally used for toxin production is the **Park-Williams 8 (PW8)**
- It is a protein having two fragments—A and B
- Enzymatic activity is present in A, and B is responsible for binding and transport of a toxin to cells

Mechanism of Action

- It acts by inhibiting protein synthesis
- Fragment—A inhibits polypeptide chain elongation in presence of NAD by inactivating elongation factor—2
- It has affinity for myocardium, nerve endings and adrenals

Synthesis of Toxin

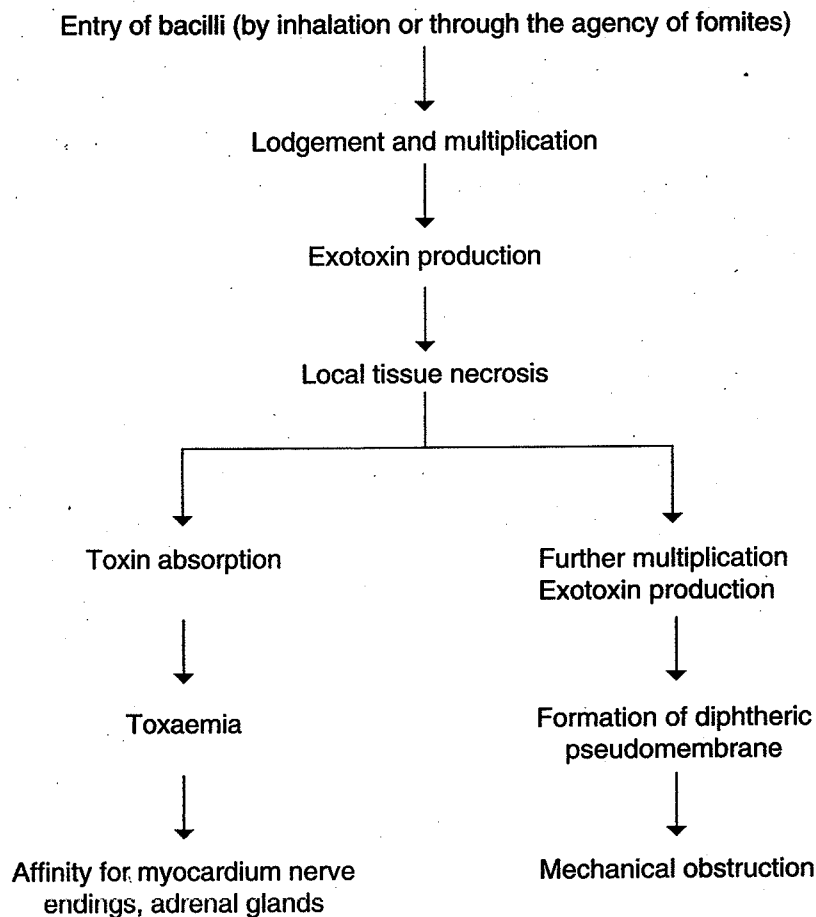
- Strains lysogenized with bacteriophages (β phage) carry 'tox' gene, which is responsible for toxin production
- Toxin production is influenced by iron in the media. Optimum iron concentration for toxin production is 0.1 mg/l of medium while iron concentration of 0.5 mg/l or more per litre is inhibitory for toxin production
- Repressor of 'tox' gene is iron-containing protein, when iron is in sufficient concentration suppressor is formed, which inhibits toxin production

■ **Diagrammatically represent the path of disease development in *C. diphtheriae* infection. Mention its clinical features.**

Pathogenesis (Flowchart 33.1)

Clinical Features

- It causes diphtheria
- Different types of diphtheria depending on sites affected are faucial, laryngeal, nasal, conjunctival, genital, cutaneous and otitic



Flowchart 33.1 Path of disease development in *Corynebacterium diphtheriae* infection.

- **Faucial diphtheria** is the commonest type
- Depending on severity, it is of three types:
 1. **Malignant:** This form shows severe toxaemia, marked adenitis, circulatory failure and high incidence of paralytic complication
 2. **Septic:** It is characterized by ulceration, cellulitis and gangrene around pseudomembrane
 3. **Haemorrhagic:** It shows bleeding from membrane, epistaxis, conjunctival haemorrhage and purpura

Complications

- Asphyxia due to mechanical obstruction
- Acute circulatory failure
- Polyneuropathy and post—diphtheritic paralysis
- Septic complications—as pneumonia and otitis media
- Diphtheric myocarditis—may terminate in heart failure and death
- Degenerative changes in adrenals, kidney and liver

■ **Discuss in brief the laboratory diagnosis of *C. diphtheriae* infection.**

Specimen

Swab from local lesion or throat swab.

Collection

Swab rubbed on membrane or lesion or post-pharyngeal wall by using tongue depressor.

Transport

It cannot be plated directly. It is moistened with sterile serum to keep bacilli viable.

Microscopic Examination

- **Gram stain**—Gram-positive bacilli arranged in Chinese letter pattern with metachromatic granules
- **Albert's stain**—green coloured bacilli with dark green metachromatic granules and Chinese letter pattern arrangement
- **Immunofluorescence**—smears are prepared from sample treated with Ab conjugated with fluorescent tag and used for detection of toxigenic diphtheria bacilli

Culture

- Specimen is plated on blood agar, blood potassium tellurite agar and Loeffler's serum slope
- Blood agar helps in differentiating it from staphylococci and streptococci
- Loeffler's serum slope shows growth in 4–8 hours; it should be incubated for 24 hours if negative for growth
- BPT is selective media incubated for 2 days
- Small, white, circular, glistening colonies from Loeffler's serum slope or gray to black colony from BPT are studied further for identification

■ **Describe the virulence tests performed to determine the pathogenicity and host tissue invasion by *C. diphtheriae*.**

Virulence Tests

- *In vivo*
 - (a) Subcutaneous test
 - (b) Intradermal test
- *In vitro*
 - (a) Tissue culture test
 - (b) Elek's test

Subcutaneous Test

- Broth emulsion of bacteria injected subcutaneously in two guinea pigs
- One guinea pig is protected with 500 units of diphtheria antitoxin 18–24 hours before the test
- If strain is virulent, unprotected animal will die within four days

Intradermal Test

- Broth emulsion inoculated intracutaneously into two guinea pigs
- One acts as control and should get 500 U of antitoxin
- The other gets 50 U of antitoxin intraperitoneally 4 hours after the test to prevent death
- Toxigenicity is indicated by inflammatory reaction at the site of injection, progressing to necrosis in 48–72 hours in test animal. Control animal does not show any change

Tissue Culture Test

- Toxigenicity of diphtheria bacilli can be demonstrated by incorporating the strain in agar overlay of cell culture monolayers
- Toxin diffuses and kills the cells below

Elek's Gel Precipitation Test

- A rectangular strip of filter paper impregnated with diphtheria antitoxin is placed on surface of 20% horse serum agar when the medium is still fluid

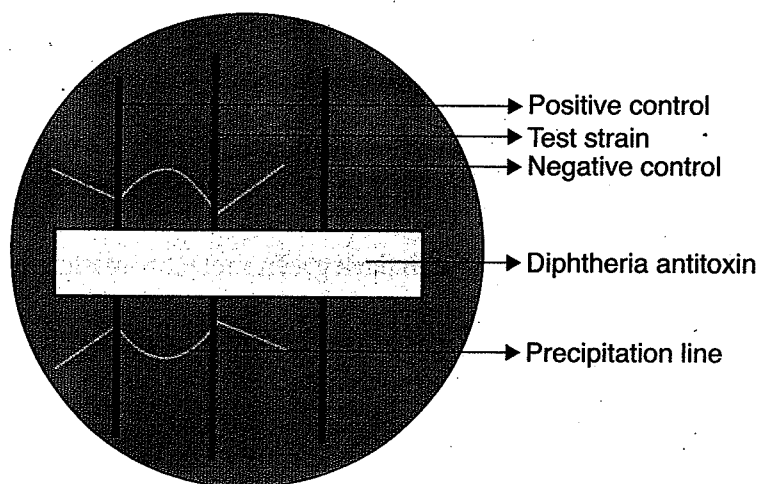


Fig. 33.3 Elek's gel precipitation test.

- When agar sets, the test strain is inoculated at right angles to the paper strip
- Positive and negative control strains are also inoculated
- Plate is incubated at 37°C for 24–28 hours
- Toxin produced by bacterial growth diffuses in agar where it meets with the antitoxin and produces a line of precipitation
- Presence of arrowhead-shaped precipitate indicates that strain is toxigenic
- Precipitation lines are not formed with nontoxigenic strains (Fig. 33.3)

■ **What treatment and prophylactic measures are adopted for combating *C. diphtheriae* infections?**

Treatment

- Penicillin and erythromycin
- Antitoxin if administered in the early stage neutralizes the toxin

Prophylaxis

- Three types – active, passive and combined immunizations
- The active immunization can provide herd immunity and lead to eradication of the disease
- The combined immunization is combination of active and passive, and used for the emergency protection

Active Immunization

- **Diphtheria, pertussis and tetanus (DPT) vaccine** contains—diphtheria toxoid, tetanus toxoid and pertussis vaccine (a killed suspension of pertussis bacilli)
- It not only minimizes the number of injections but also improves immune response as pertussis vaccine acts as an adjuvant for diphtheria and tetanus toxoids
- **Schedule**—3 doses. The first dose of DPT is given at the age of 6 weeks (as antibodies received passively from mother protect the children till then). The second and third doses are given at an interval of 4 weeks (10th and 14th week and fourth dose at 18th month of age) and 2nd booster at 5–6 years
- As diphtheria and pertussis are uncommon after 5 years, only booster dose of tetanus is given at school entry and thereafter
- **Route:** It is given intramuscularly
- **Type of immunity**—antibody mediated (humoral immunity)

Passive Immunization

It is an emergency measure, used only once when susceptible person is exposed to a known case of diphtheria. Subcutaneous administration of 500–1000 units of antitoxin–antidiphtheric serum (ADS) is recommended.

Combined

- It consists of dose of adsorbed toxoid on one arm and ADS on the other arm. It should be followed by complete immunization schedule
- Alum containing preparations are preferred over formal toxoid as its response is unsatisfactory when given with ADS

■ Write in short about diphtheroids.

- Commensal corynebacteria morphologically similar to *C. diphtheriae* normally present in the throat, skin, conjunctiva and other areas are known as diphtheroids.
- Because of morphological similarity they may be mistaken for diphtheria bacilli.
- The common diphtheroids include *C. xerosis* (normally present in the conjunctival sac) and *C. pseudodiphtheriticum*/*C. hofmannii* (normally present in the throat).
- The distinguishing features of *C. diphtheriae* and diphtheroids are given in Table 33.2.

Table 33.2 The distinguishing features of *C. diphtheriae* and diphtheroids

1. Feature	<i>C. diphtheriae</i>	Diphtheroids
a. Morphology	1. Weakly gram-positive and thin bacilli 2. Metachromatic granules- present 3. Arrangement- Chinese letter pattern	1. Strongly gram-positive, short and thick bacilli that stain more uniformly 2. Metachromatic granules- few or absent 3. Pallisade Arrangement
b. Culture	Requires special enriched media for growth	Can grow on ordinary media
c. Biochemical reactions	Ferments glucose	Ferment glucose and sucrose
d. Toxicity	Toxic	Nontoxic

34

Chapter

Bacillus

■ Narrate the historical importance of *Bacillus*.

- First pathogenic bacteria observed under microscope was *B. anthracis*. This observation was made by Pollander
- Robert Koch formulated the well known Koch's postulates on the basis of his observations on anthrax
- First bacteria isolated in pure culture was *B. anthracis*. The isolation was performed by Koch
- First communicable disease shown to be transmitted by infected blood was anthrax
- Pasteur developed first bacterial vaccine by using *B. anthracis*
- Germ theory of disease was established with *B. anthracis*

■ Mention the practical importance of *Bacillus* spp.

Bacillus spp. can be used in the following ways:

- As controls in sterilization procedures
 - *B. steurothermophilus* strips in autoclave
 - *B. subtilis* strips in hot air oven
- To produce antibiotics
 - *B. polymyxa*—polymyxin
 - *B. subtilis*—bacitracin
- As a model in the study of genetics, e.g. *B. subtilis*
- For pest control on food crops, e.g. *B. thuringiensis*

■ State the characteristic morphological features of *B. anthracis*.

Morphological Features

- Large Gram-positive bacillus, about $4-8 \mu \times 1.0-1.5 \mu$ in size
- In tissues—it is arranged in chains, pairs or singly
- In culture—it is arranged in long chains, ends of bacilli are swollen and truncated giving "bamboo-stick" appearance
- Possess spores, which are oval central and nonbulging (formed in culture or soil)
- Nonmotile
- Entire chain is surrounded by polypeptide capsule

M'Fadyean's Reaction

Blood films containing *B. anthracis* when stained with polychrome methylene blue and examined under microscope show an amorphous purplish material around bacillus, which represents capsular material and is a characteristic feature of *B. anthracis*.

Giemsa stained smear shows a red capsule around purple bacillus (Fig. 34.1).

- **Mention the cultural characteristics of *B. anthracis* include media and colony characters.**

Cultural Characters

- Aerobe and facultative anaerobe
- Optimum temperature 37°C (range, 12°–45°C) and optimum pH 7–7.4
- Can grow on ordinary media

Media and Colony Characters

Nutrient agar

Large, 2–3 mm in diameter, raised, dull, opaque, gray-white colony with irregular edge. Under low power of microscope colony gives “Frosted glass appearance” and edge of colony shows ‘Medusa head appearance’, i.e. long interlacing chain of bacilli resembling curled matted hair.

Blood agar

The colonies are generally non-haemolytic, but occasional strains may produce a narrow zone of haemolysis.

Selective media

PLET media—containing heart infusion agar with polymyxin, lysozyme, ethylene diamine tetra-acetic acid and thallos acetate.

Gelatin stab culture

Inverted fir tree appearance of colony with slow liquefaction commencing from top.

String of pearls reaction

On solid media containing 0.05–0.5 U of penicillin/ml, cells become large, spherical in 3–6 hours and appear in chain on surface of agar.

- **Mention biochemical reactions of *B. anthracis*.**

Biochemical Reactions

Not important in identification

- Ferments many sugars with acid production
- Catalase and nitrate reduction are positive

- **In what ways can *B. anthracis* be destroyed?**

- In vegetative state, bacilli are sensitive to physical and chemical agents; however, spores are highly resistant to both these agents
- Spores may remain viable in soil for about 60 years
- Dry heat at 140°C for 1–3 hours and boiling kills the spores
- “Duckering” results in spore destruction in animal products where 2% formaldehyde at 30–40°C for 20 minutes is used for wool and 0.25% at 60°C for 6 hours for animal hairs and bristles

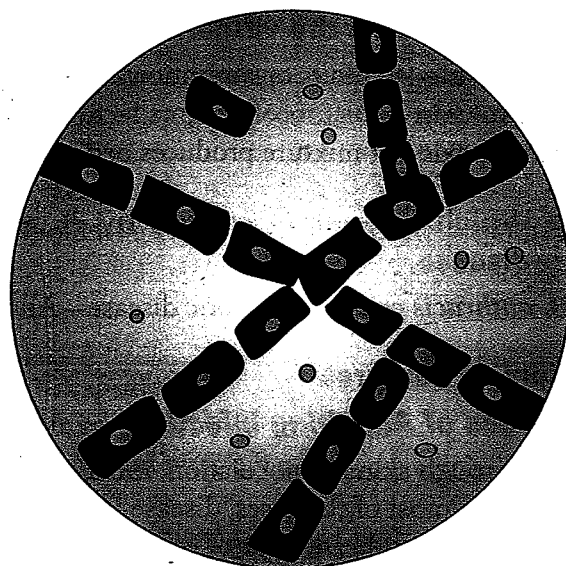


Fig. 34.1 *Bacillus anthracis*.

■ **What marks the virulence in *B. anthracis*?**

- **Capsule**—inhibits opsonization and phagocytosis
- **Exotoxin**—consists of oedema factor, protective antigen factor and lethal factor. Individually nontoxic but mixture produces oedema and shock

■ **Which disease is caused by *B. anthracis*? Give the clinical features of the disease.**

B. anthracis causes a zoonotic disease—**Anthrax**

Clinical Features

Modes of Acquiring Infection

- Through skin by inoculation, inhalation and ingestion
 - In most of the cases, disease is contracted as an occupational hazard due to contact with meat, hide and hair of infected animals
 - In others it is due to handling of infected animal products such as shaving brushes and leather goods

Clinical Types

1. Cutaneous anthrax
2. Pulmonary anthrax
3. Intestinal anthrax

Cutaneous Anthrax

- Common in dock workers carrying loads of hides and skin on their back; it is therefore called "**Hide porters disease**." It is also called **malignant pustule**. Infection is acquired by inoculation of spores through skin
- Sites—face, neck, hand, arms and back
- Lesion—starts as papule, becomes vesicular, containing clear or blood-stained fluid. Infected area becomes oedematous and congested, leading to necrosis at centre covered by black eschar. Satellite lesion appear surrounding the central lesion
- Complications—untreated patients develop fatal septicaemia or meningitis

Pulmonary Anthrax

- Common in wool factory workers
- Also called "**wool sorter's disease**"
- Infection is acquired due to inhalation of spores from infected wool
- Manifest as—Haemorrhagic pneumonia
- Complication—Haemorrhagic meningitis

Intestinal Anthrax

- Rare form of anthrax
- Found in community who eat improperly cooked infected meat
- Clinically, patient presents with bloody diarrhoea

■ **Mention the laboratory diagnosis of *B. anthracis* infection.**

Specimens

Blood, sputum, fluid or pus from malignant pustules, gastric aspirate, and stool sample.

Collection

With all handling precautions, fluid is aspirated from local lesions, blood is collected by venepuncture and added to glucose broth and sputum is collected in a dry, leak-proof sterile container.

Transport

Blood is transported in glucose broth processing should be carried out in Biosafety cabinets with all precautions.

Microscopic Examination

- **Gram-stained smears**—Gram-positive bacilli with capsule
- **India ink**—Clear halo around bacteria represents capsule
- **Giemsa-stained smears**—They show a red capsule around purple bacillus
- **Direct fluorescent antibody test**—For capsule
- **M'fadyean's reaction**—Blood films containing *B. anthracis* when stained with polychrome methylene blue and examined under microscope show an amorphous purplish material around bacillus, which represents capsular material and is a characteristic feature of *B. anthracis*

Culture

- Specimen is inoculated on nutrient and blood agar and selective medium—PLET
- Nutrient agar is observed for large, 2–3 mm in diameter, raised, dull, opaque, gray-white colony showing “Frosted glass appearance” and ‘Medusa head appearance’ is processed further for identification

Biochemical Reactions

- Catalase and gelatin liquefaction—Positive

Animal Inoculation

- Intraperitoneal inoculation of sample in mice or guinea pig
- Blood smear observed after death of animal for bacilli

Antibody Detection Tests

- ELISA
- Indirect haemagglutination test

Antigen Detection Test

Ascoli's thermoprecipitation test to detect Ag in tissue.

Nucleic Acid Detection

Polymerase chain reaction

- Give the treatment and prophylactic measures adopted for combating *B. anthracis* infection.

Treatment

- Penicillin
- Streptomycin
- Doxycycline
- Ciprofloxacin

Prophylaxis

Alum precipitated toxoid prepared by using protective Ag

- Used in occupational risk groups
- Schedule—3 doses intramuscularly, at interval of 6 weeks between 1st and 2nd dose, and 6 months between 2nd and 3rd dose

■ Differentiate between anthrax and anthracoid bacilli.

Features distinguishing anthrax bacilli from anthracoid bacilli are enumerated in Table 34.1.

Table 34.1 Differences between anthrax and anthracoid bacilli

Character	Anthrax	Anthracoid
1. Morphology		
a. Capsule	Capsulated	Noncapsulated
b. Motility	Nonmotile	Motile
c. Length of chain	Long chains	Short chains
d. M'Fadyean's reaction	Positive	Negative
2. Growth characters		
a. On Nutrient agar	Medusa head colony	Not typical
b. On Blood agar	Haemolysis weak	Marked haemolysis
c. Gelatin stab culture	Inverted fir tree appearance with slow liquefaction	Rapid liquefaction
d. Broth	No turbidity	Turbidity
e. Growth at 45°C	Does not grow	Grows
f. Growth on penicillin agar	No growth	Grows
3. Identification tests		
a. Ascoli's precipitation test	Positive	Negative
b. Susceptibility to gamma phage	Susceptible	Not susceptible
4. Pathogenicity to guinea pig	Pathogenic	Not pathogenic

■ How does *B. cereus* cause infection?

- *B. cereus* is an anthracoid bacillus
- It is an important agent causing food poisoning
- It causes toxin type of food poisoning
- When food is stored at warm temperature, spores germinate into vegetative bacilli, multiply and elaborate toxin-causing food poisoning

■ Name the clinical types of *B. cereus* infection.

Clinical types of *B. cereus* infection are

1. Emetic form and
2. Diarrhoeal form

■ Write differentiating features between emetic and diarrhoeal types of food poisoning.

Differences between emetic and diarrhoeal types of food poisoning are presented in Table 34.2.

Table 34.2 Differences between emetic and diarrhoeal types of food poisoning

	<i>Emetic type</i>	<i>Diarrhoeal type</i>
1. Food	Fried rice (from Chinese restaurant)	Cooked meat, vegetables
2. Incubation period	1–5 hours	8–46 hours
3. Clinical features	Acute nausea vomiting Diarrhoea not common	Abdominal pain Vomiting not common
4. Serotypes responsible	1, 3, 5	2, 6, 8, 9, 10, 12
5. Mechanism of action	Emetic toxin production	Enterotoxin causes fluid Accumulation

■ Which laboratory diagnostic tests are recommended for detection of *B. cereus* infection?

- Isolation of bacteria from stool or food by usual methods using mannitol egg-yolk-phenol red polymyxin (MYPA) agar
- Demonstration of toxin

Clostridium

■ Explain briefly the general features of clostridia.

- The anaerobes are differentiated into two groups based on formation of spores
 1. Spore forming anaerobes
 2. Nonspore forming anaerobes
- Spore forming anaerobes are included in the genus *Clostridium*. These are:
 - Gram-positive bacilli
 - Spindle shaped because of bulging spore (the name *Clostridium* is derived from the word Kloster, meaning spindle)
 - Most species are motile with peritrichous flagella except *Cl. welchii*
 - Noncapsulated except *Cl. welchii*
 - Widely distributed in nature—saprophytes—soil is the main habitat
 - Some pathogens, e.g. *Cl. welchii* and *Cl. tetani* are commensal in intestinal tract of man and animals

■ Classify clostridia on the basis of the diseases produced by them.

Based on diseases they produce in human beings, clostridia have been classified into 4 types (Table 35.1).

Table 35.1 Classification of clostridia based on human pathogenicity

Group	Species
1. Tetanus	<i>Cl. tetani</i>
2. Food poisoning	
• Gastroenteritis	<i>Cl. perfringens</i> (Type A)
• Botulism	<i>Cl. botulinum</i>
• Necrotizing enteritis	<i>Cl. perfringens</i> (Type C)
3. Gas gangrene	<i>Cl. perfringens</i>
	<i>Cl. septicum</i>
	<i>Cl. novyi</i>
	<i>Cl. histolyticum</i>
	<i>Cl. fallax</i>
	<i>Cl. bifermentans</i> *
	<i>Cl. sporogenes</i> *
4. Acute colitis	<i>Cl. difficile</i>

*Doubtful pathogens.

■ Name the scientist after whom *Cl. welchii* is so-called.

Cl. welchii was described by Welch and Nuttall in 1892; hence, it is named as *Cl. welchii*. It is also known as *Clostridium perfringens*.

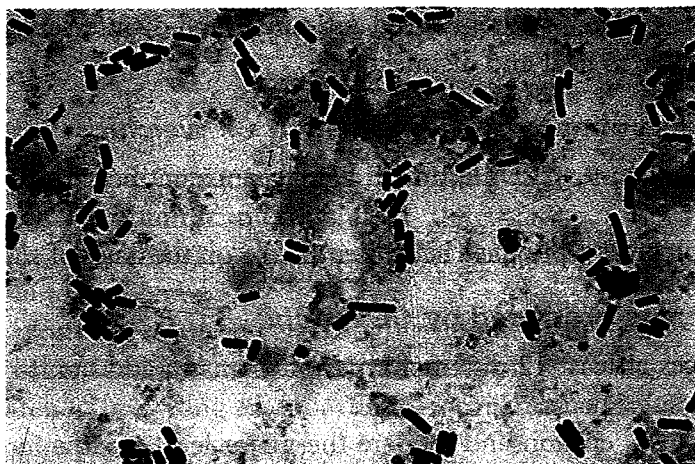


Fig. 35.1 *Clostridium welchii*. (Source: Mosby's Dictionary of Medicine, Nursing & Health Professions, Pages 265-479, Elsevier, 2010.)

■ **State the salient morphological features of *Cl. welchii*.**

Morphological Features (Fig. 35.1)

- Gram-positive bacilli with parallel sides and round ends
- Size: $4-6 \mu \times 1 \mu$
- Arranged singly, in chains, and in bundles
- Spores: Large bulging oval and subterminal
- Rarely seen in artificial culture and in direct smears from tissues
- Capsulated but nonmotile

■ **Mention the cultural characteristics of *Cl. welchii*; include media and colony characters.**

Cultural Characters

- Anaerobic, but can grow under microaerophilic condition
- Temperature 20–50°C, optimum 45°C
- pH 5.5–8.0, optimum 7.2–7.4
- Sporulation is favoured in alkaline medium

Media and Colony Characters

- **Media:** Blood agar and Robertson's cooked meat broth (RCM)
- **Blood agar:** It grows producing a target haemolysis. Narrow zone of complete haemolysis due to theta toxin and wider incomplete haemolysis due to alpha toxin
- **RCM:** It grows rapidly, turning meat particles pink in colour but there is no digestion of meat particles (saccharolytic activity)

■ **State the biochemical reactions that help in detection of *Cl. welchii*.**

Biochemical Reactions

- It ferments glucose, lactose, sucrose and maltose with acid and gas
- It is indole-negative, methyl red (MR) positive, Voges-Proskauer (VP)—negative and H_2S —positive
- Most strains reduce nitrate to nitrites

■ **Mention the agents to which *Cl. welchii* is sensitive or resistant?**

- Spores are destroyed by boiling for 5 minutes except type A and C strains, which are markedly resistant to boiling for 1–3 hours
- Autoclaving at 121°C for 15 minutes destroys spores

- Spores are also resistant to commonly used antiseptics and disinfectants, however vegetative cells are very sensitive to heat and disinfectants

■ **Mention the types of antigens identified in *Cl. welchii*.**

- Six antigenic types—A, B, C, D, E, and F have been identified on the basis of the toxin produced by them
- More than 80 serological types have been identified—on the basis of surface polysaccharide

■ **Describe the toxins produced by *Cl. welchii*.**

- *Cl. welchii* produces at least 12 different types of toxins in addition to many enzymes and biologically active substances
- Out of 12, alpha, beta, epsilon and iota are the major lethal toxins for mice and form the basis of classification of *Cl. welchii* into A to F. They are also responsible for pathogenicity

Alpha Toxin

- It is more important toxin produced by all antigenic types of *Cl. welchii*
- It is lethal, dermonecrotic and haemolytic
- It is responsible for toxæmia of gas gangrene
- Chemically, it is a phospholipidase (lecithinase C) that splits lecithin (an important constituent of cell membrane) to phosphoryl choline and a diglyceride. This activity can be demonstrated by Nagler reaction
- The beta, epsilon and iota toxins have lethal and necrotizing properties
- Some other toxins and soluble substances, which are important in pathogenicity, include:
 - Theta-toxin—haemolytic and necrotizing
 - Deoxyribonucleases (nu toxin)
 - Hyaluronidase (mu toxin)
 - Collagenase (kappa toxin)
 - Haemolysin
 - Fibrinolysin

Enterotoxin

- Some strains of type A produce powerful enterotoxin, especially when they grow in meat dishes
- It is a heat labile protein (molecular weight 36,000) that is formed in large intestine during sporulation and appears to be identical to a component of spore coat
- It causes hypersecretion in the jejunum and ileum leading to loss of fluids and electrolytes
- It induces intense diarrhoea in 6–18 hours

■ **Enumerate the diseases caused by *Cl. welchii*.**

Cl. welchii causes the following infections:

- Gas gangrene
- Food poisoning
- Necrotizing enteritis
- Gangrenous appendicitis
- Brain abscess and meningitis
- Urogenital infections
- Panophthalmitis
- Biliary tract infections
- Septicaemia and endocarditis
- Anaerobic cellulitis

■ Discuss the pathogenesis of gas gangrene and food poisoning.

Pathogenesis

Gas Gangrene

- Organisms enter the tissue by contamination of traumatized areas from foreign particles such as soil, dust, etc. or from the intestinal tract
- Presence of foreign body and growth of facultative anaerobes, create anaerobic conditions allowing *Cl. welchii* to proliferate
- The proliferating clostridia release necrotizing toxin and hyaluronidase causing devitalization of adjacent tissues and interference with blood supply
- Tissue necrosis extends, providing an opportunity for increased growth of the organism and release of more toxins and enzymes
- This damage progresses leading to severe toxæmia and death

Food Poisoning

- Spores resistant to heat survive the cooking process, germinate and increase in number in warm meat
- When ingested along with food, bacteria grow in intestine, form and release toxin when they sporulate in gut and thereby produce symptoms of food poisoning

■ Write the clinical features of gas gangrene and food poisoning by *Cl. welchii*.

Clinical Features of Gas Gangrene

Local symptoms

- Swelling of infected tissue
- Crepitation in the subcutaneous tissue and muscle
- Foul-smelling discharge—dark fluid
- Gas formation causing distension of subcutaneous tissue and pain
- Rapidly spreading necrosis

Generalized symptoms

Fever, toxæmia, shock and death.

Food Poisoning

Symptoms include:

- Diarrhoea without vomiting
- Cramps and abdominal pain
- The illness lasts for 1–2 days

■ Describe the laboratory diagnosis of (a) gas gangrene and (b) food poisoning.

(a) Laboratory Diagnosis of Gas Gangrene

Specimens

- Material from wound, exudates, excised or necrosed tissue
- Specimens should be collected from deeper sites

Transport

To avoid death of anaerobes, sample is to be collected and transported in RCM medium or thioglycollate broth

Direct Microscopy

- Gram-stained smear helps in presumptive diagnosis of gas gangrene. In case of infection by *Cl. welchii*, Gram stain shows large number of Gram-positive bacilli without spores and scanty pus cells, which is a characteristic feature of gas gangrene
- *Cl. septicum* appears as—citron bodies and boat- or leaf-shaped pleomorphic bacilli
- *Cl. novyi*—large bacilli with oval subterminal spores

Culture

- The specimen is inoculated on fresh blood agar, neomycin blood agar and RCM medium
- One blood agar plate and neomycin blood agar are incubated anaerobically at 37°C for 48–72 hours
- One blood agar is incubated aerobically
- The cultures are observed for typical colonies and subjected for identification using following tests

Biochemical Reactions and Other Tests for Identification

1. Litmus milk test
2. Nagler reaction

Litmus Milk Test

- In litmus milk, *Cl. welchii* changes colour of litmus from blue to red because of acid formation from fermentation of lactose. The acid produced coagulates casein leading to formation of acid clot, and this clotted milk is disrupted due to vigorous gas production. This is known as stormy fermentation. Disruption of clot by gas in 24 hours is suggestive of *Cl. welchii*

Nagler Reaction (Fig. 35.2)

- The test is devised by Nagler
- The test is performed on agar medium containing 20% human serum or 5% egg yolk

Principle

- *Cl. welchii* produces a zone of opacity surrounding the colonies in media containing serum or egg-yolk due to lecithinase activity (phospholipase) of alpha toxin that causes deposition of lipid around the colony-producing zone of opacity

Procedure

- The test bacterium is grown on a medium
- To one half of the plate, antitoxin is spread on the surface
- Plate is incubated at 37°C in anaerobic condition for 24 hours

Results

- Colonies on the side containing no antitoxin show a zone of opacity while the colonies on the other half with antitoxin show no opacity due to neutralization of alpha toxin by antitoxin
- Positive test confirms the identity of *Cl. welchii*

Animal Pathogenicity

- 0.1 ml of 24 hours growth in RCM is injected intramuscularly on hind limb of guinea pig

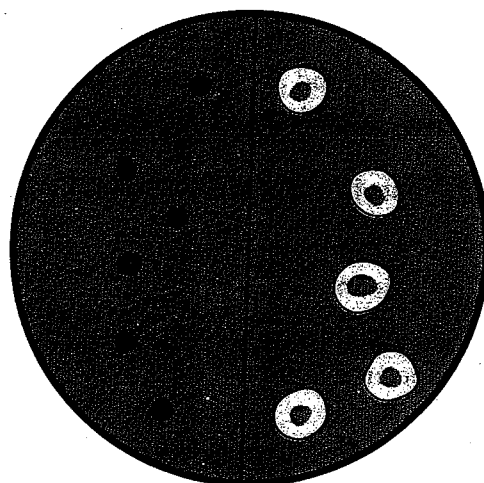


Fig. 35.2 Nagler reaction.

- Death occurs within 24 hours
- On autopsy *Cl. welchii* can be recovered from heart and spleen

(b) Laboratory Diagnosis of Food Poisoning

Specimens

- Faeces and suspected food articles
- Specimen is collected in RCM medium and subcultured on selective medium and incubated anaerobically
- The typical colonies—identified by morphology, cultural characters, biochemical reactions and Nagler reaction. The procedure is same as in gas gangrene
- As *Cl. welchii* may be normally present in intestine, hence isolation from faeces in large number is meaningful

■ Mention the treatment strategy for gas gangrene and food poisoning.

Methods of treating gas gangrene and food poisoning are:

- Prompt and extensive surgical debridement of the involved area and removal of dead tissue, wound irrigation and treatment with hyperbaric oxygen
- Antibiotic treatment, particularly Penicillin administration immediately
- Passive immunization with antitoxin containing concentrated immunoglobulins
- Treatment of food poisoning requires only symptomatic care

■ Who discovered *Clostridium tetani* and who isolated it first?

Clostridium tetani was first demonstrated by Rosenbach 1886 from a case of tetanus. Kitasato (1889) isolated it in pure culture and reproduced the disease in experimental animal.

■ Mention the distinguishing morphological features of *Cl. tetani*.

Morphological Features

- Gram-positive straight, slender bacilli
- Size: $2-5 \mu \times 0.4-0.5 \mu$
- Larger filaments may occur
- Motile by peritrichous flagella except type VI
- Noncapsulated
- Sporing: Spore formed is terminal, spherical, bulging
- Spore gives drumstick appearance to the bacilli (Fig. 35.3)

■ Mention the cultural characteristics of *Cl. tetani*, include media and colony characters.

Cultural Characters

- Obligate anaerobe
- Optimum temperature 37°C and optimum pH 7.2–7.4

Media and Colony Characters

- **Media:** Blood agar, Robertson cooked meat medium (RCM) and thioglycollate broth

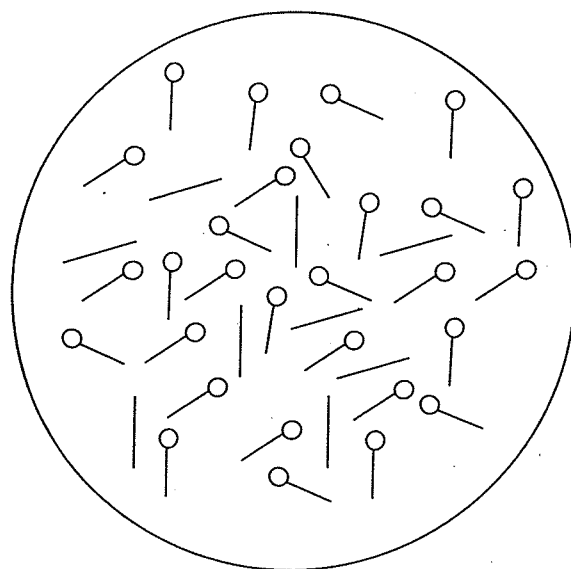


Fig. 35.3 *Clostridium tetani*.



Fig. 35.4 *Clostridium tetani*—swarming growth.

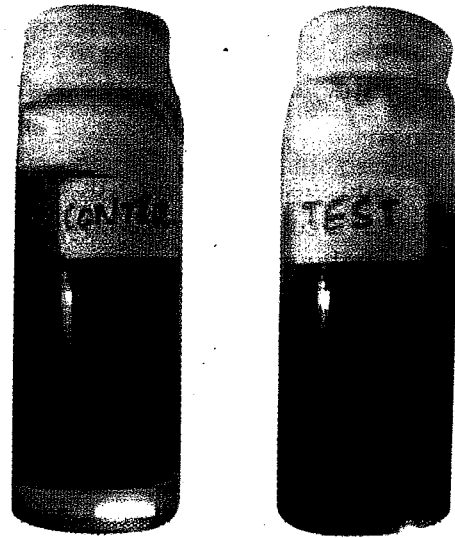


Fig. 35.5 *Clostridium tetani*—proteolytic activity.

- **Blood agar:** Swarming, spreading type of growth with alpha-haemolysis followed by beta-haemolysis (Fig. 35.4)
- **Agar stab culture:** Fir tree appearance
- **RCM:** It becomes turbid, meat is not digested but becomes slightly blackened, showing slight proteolytic effect (Fig. 35.5).

■ Which biochemical reactions indicate the presence of *Cl. tetani*?

Biochemical Reactions

- Proteolytic in nature
- Does not attack sugars
- Forms indole
- Methyl red, Voges-Proskauer and citrate—negative
- H_2S —not formed
- Gelatin liquefaction—negative
- Nitrate reduction—negative

■ In what ways can *Cl. tetani* be killed?

- *Cl. tetani* can usually be killed by boiling for 10–15 minutes
- Autoclaving ensures destruction of spores
- Spores are resistant to most antiseptics
- 1% iodine and hydrogen peroxide kill the spores in few hours

■ On what basis is *Cl. tetani* classified?

- Based on flagellar Ag (H Ag)—*Cl. tetani* is typed into 10 serotypes by agglutination reaction. These 10 types are named as I to X
- Type VI—are nonflagellated strains

■ Mention the toxins produced by *Cl. tetani*. Give prominent features of each.

Toxins produced by *Cl. tetani* are

- Tetanolysin
- Tetanospasmin (neurotoxin)

Tetanolysin

Heat labile and oxygen labile haemolysin, pathogenic role is unknown

Tetanospasmin

Heat labile and oxygen stable powerful neurotoxin. Protein in nature. It is a heterodimer consisting of a large polypeptide chain and a smaller polypeptide chain linked together by a disulphide bond. It is antigenic and specifically neutralized by antitoxin. It can be toxoided.

■ **Under which conditions does *Cl. tetani* develop infection. Mention the predisposing factors.**

Pathogenicity- *Cl. tetani* is the cause of tetanus

- Tetanus occurs following
 - Injury
 - Unsterile injections
 - Rituals—such as circumcision or ear boring
 - Septic abortion
 - Dirty practices such as application of cow dung on the umbilical stumps

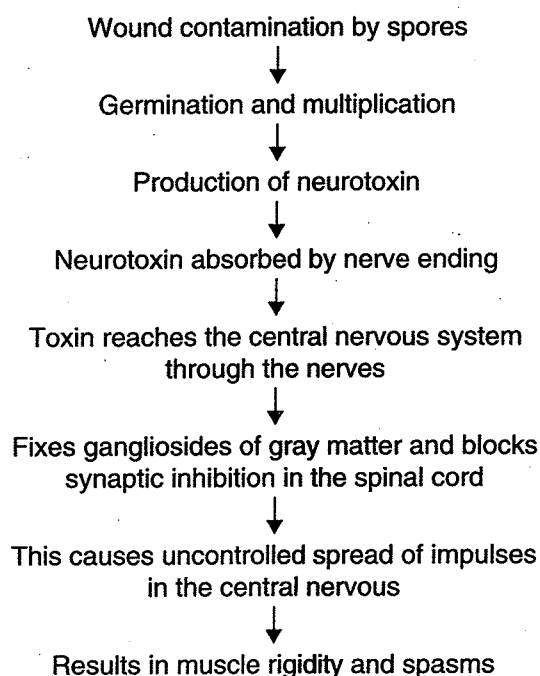
■ **In the form of a flowchart represent the mechanism of disease development in *Cl. tetani* infections.**

The path of disease development is diagrammatically represented in Flowchart 35.1.

■ **Describe the clinical features.**

Clinical Features of *Cl. tetani* infections are:

- Incubation period: Two to several weeks (commonly 6–12 days). It is influenced by site and nature of wound, dose and toxigenicity of an organism and immune status of host
- Experimental tetanus is of three types: local, ascending and descending
 - Local type—develops spasm at the site of injection



Flowchart 35.1 Mechanism of disease development in *Clostridium tetani* infections.

- Ascending type—spreads to spinal cord from local site
- Descending type—resembles natural tetanus
- When toxin is injected intravenously, spasm develops in head-neck and then spreads downwards
- Tonic muscle spasms at the site of infection becomes generalized involving whole of somatic muscular system
- Spastic paralysis involves jaw muscles (**lock jaw**)

Other types

- Tetanus neonatorum
- Uterine tetanus
- Otogenic tetanus

■ What laboratory diagnostic methods should be adopted for identifying *Cl. tetani*?

Specimens

Necrotic tissue from depth of wound, exudates from wound or wound swab.

Collection

Necrotic tissue bits excised and collected in anaerobic transport media. Alternatively, exudates from wound, wound swab may be collected and transported in anaerobic transport medium.

Microscopy

Demonstration of *Cl. tetani* is unreliable, as nonpathogenic clostridia such as *Cl. tetanomorphum* and *Cl. sphenoides*, morphologically similar to *Cl. tetani* may be present normally in specimen.

Culture

Best and confirmatory method

- **Media:** Blood agar, Robertson's cooked meat medium (RCM), thioglycollate broth
- **Blood agar:** Swarming-spreading type of growth with alpha-haemolysis followed by beta-haemolysis
- **Agar stab culture:** Fir tree appearance
- **RCM:** becomes turbid, meat not digested but gets slightly blackened, exhibiting slight proteolytic effect
- Three RCM bottles are used—first bottle heated at 80°C for 15 minutes, second bottle heated at 80°C for 5 minutes to kill vegetative forms and third bottle left unheated, incubated at 37°C for 24–48 hours, subcultured on BA observed for growth

■ Describe the procedure(s) for toxigenicity testing of *Cl. tetani*.

- Toxigenicity testing on horse blood agar
 - On horse blood agar having antitoxin spread over half of the plate, *Cl. tetani* stab is inoculated on each half of the plate
 - Incubated anaerobically for 24–48 hours and observed for haemolysis around colony
 - Haemolysis is seen on the half plate without antitoxin and haemolysis is inhibited on the other half
 - Test—detects tetanolysin, which is also produced by nonpathogenic strains, hence not reliable
- Toxigenicity testing by animal inoculation
 - Two mice are inoculated with 0.2 ml broth suspension into root of tail
 - One is protected with 1000 U of antitoxin that serves as control

- Test animal develops stiffness of tail, rigidity of leg on inoculated site, followed by opposite side leg, trunk and forelimbs—dies in 2 days
- It detects tetanospasmin, which is produced by pathogenic strains, hence it is reliable
- Tetanospasmin can also be detected by PCR

■ Mention the treatment strategy for tetanus.

Treatment strategy for tetanus includes:

- Isolation—for protection from noise and light
- Human anti-tetanus immunoglobulin (TIG) followed by full course of active immunization
- Control of spasms
- Maintenance of air way by tracheostomy
- Attention to feeding
- Antibacterial therapy—Penicillin or metronidazole
- Full course of active immunization in patients recovering from tetanus

■ What prophylactic measures should be adopted for prevention of *Cl. tetani* infection?

Prophylactic measures that should be adopted for prevention of *Cl. tetani* infection are:

1. **Surgical**—removal of foreign body, necrotic tissue and blood clots to prevent development of anaerobic environment
2. **Antibiotic prophylaxis**—if administered in 4 hours, may destroy or inhibit bacilli, and toxin production is prevented
 - Penicillin and tetracycline are effective
 - Bacitracin or neomycin applied—locally
3. **Immunization** (Table 35.2)—active, passive and combined

Table 35.2 Prophylaxis for tetanus

Wound type	Immune persons	Partially immune	Nonimmune
Clean	1 dose of toxoid	1 dose of toxoid	3 doses of toxoid
Contaminated	1 dose of toxoid	1 dose of toxoid + TIG + antibiotics	3 doses of toxoid + TIG + antibiotics
Infected	1 dose of toxoid + antibiotics	1 dose of toxoid + TIG + antibiotics	3 doses of toxoid + TIG + antibiotics

Active immunization

- Formal toxoid or toxoid absorbed on aluminium phosphate or hydroxide. Also given as triple vaccine DPT
- Toxoid—3 doses—intramuscularly at an interval of 4–6 weeks, between 1st and 2nd doses and 6 months between 2nd and 3rd dose
- Immunity lasts for 10 years. Booster after 10 years or after 3 years if wounding occurs

Passive immunization

- Anti-tetanus serum (ATS) or human anti-tetanus immunoglobulin
- Recommended only in nonimmune persons at risk and only once
- ATS—1500 IU or Human anti-tetanus immunoglobulin TIG—250 IU
- Disadvantages of ATS
 1. Immune elimination
 2. Hypersensitivity

Combined immunization

TIG on one hand and toxoid on another site (hand) followed by 2nd and 3rd dose of toxoid.

■ How did *Cl. botulinum* acquire its name?

- *Cl. botulinum* is the cause of botulism—a severe form of food poisoning presenting as paralytic disease
- The term botulism means sausage, as poorly cooked sausages were formerly associated with this type of food poisoning

■ State the characteristic morphological features of *Cl. botulinum*.**Morphological Features**

- Gram-positive bacilli
- Size: $4-6\ \mu \times 1\ \mu$
- Motile by peritrichous flagella
- Noncapsulated
- Spore bearing—forms subterminal, oval, bulging spores

■ Mention the cultural characteristics of *Cl. botulinum*. Also, mention the media and colony characters.**Cultural Characters**

- Strict anaerobe
- Optimum temperature 35°C , can grow at $1^{\circ}-5^{\circ}\text{C}$
- Can grow on ordinary media such as nutrient agar

Media and Colony Characters

- **Media:** Blood agar, RCM, nutrient agar, lactose egg yolk milk agar
- **Colony:** It develops after 48 hours incubation
- **Nutrient agar:** It forms 3–8 mm, irregular, semitransparent colonies with fimbriate edges
- **Blood agar:** Colonies are haemolytic
- **RCM medium:** After 2–4 days, it causes blackening of meat particles and production of gas
- **Lactose egg yolk milk agar medium:** It produces opalescence and pearly layer

■ Which biochemical reactions are specific to *Cl. botulinum*?

Biochemical reactions specific to *Cl. botulinum* are, as follows:

- Ferment glucose and maltose with production of acid and gas
- Produce H_2S
- Biochemically two types:
 - Proteolytic—Type A, B and F
 - Saccharolytic—Type C, D and E

■ To which factors is *Cl. botulinum* resistant?

- Spores—highly resistant—can survive at 100°C for several hours
- Spores of type B, E and F are less resistant
- Also resistant to radiation, alcohols, phenols and quaternary ammonium compounds
- Relatively susceptible to hypochlorite, ethylene oxide and formaldehyde

■ Name the antigenic component of *Cl. botulinum*. How is it classified?

Toxin is antigenic. Based on its antigenicity, seven antigenically different types of toxins have been identified—A to G.

■ **Describe the nature of the toxin produced by *Cl. botulinum* and mention its mode of action.**

- The toxin produced by *Cl. botulinum* is an exotoxin, protein in nature
- It differs from other known exotoxins in that it is released on the death and autolysis of the organism and not during the life of organism
- It is highly potent—1 mg is enough to kill 1 million guinea pigs
- It is a neurotoxin that acts slowly
- The toxin acts by binding specifically to the motor nerve plates and blocks the calcium-mediated release and production of acetylcholine at the synapses and neuromuscular junctions, preventing the contraction causing flaccid paralysis
- It is relatively heat stable and resistant to intestinal digestion. Destroyed only after 30–40 minutes at 80°C and in 10 minutes at 100°C
- Boiling for 20 minutes destroys toxin in foodstuff

■ **Describe the pathogenicity of *Cl. botulinum*.**

- *Cl. botulinum* is noninvasive and noninfectious and its pathogenicity is due to toxin
- It causes botulism, which is of three types:
 1. Food-borne botulism
 2. Wound botulism
 3. Infant botulism

Food-borne botulism

- Infection occurs by ingestion of preformed toxin in canned meat and meat products, canned vegetables such as mushrooms, green beans, pepper, etc. and smoked fish
- Spores contaminate vegetables and meat
- When these foods are canned or vacuum packed with inadequate sterilization—spores germinate and form toxin in canned food (preformed toxin)
- Symptoms begin after 12–36 hours after ingestion
- Symptoms are:
 - Vomiting, thirst, constipation, ocular paresis, difficulty in swallowing (dysphagia) and respiratory muscle failure leading to death in 1–7 days
 - It is fatal; fatality rate is 20–70%

Wound botulism

- Infection of wound with spore, germination and production of toxin at the site leads to wound botulism
- Symptoms are same as in food-borne botulism, except for gastrointestinal symptoms.

Infant botulism

- It occurs in infants below 6 months
- Infection occurs by ingestion of food containing spores, organisms grow in the gut and produce toxins
- Ingestion of honey-containing organisms is most likely the cause
- Infant botulism is characterized by constipation, poor feeding, lethargy, weakness, pooled oral secretions, weak or altered cry, floppiness and loss of head control

■ **How is laboratory diagnosis of *Cl. botulinum* infection performed?**

Specimens

Faeces and food

Microscopy

Gram stain—may show Gram-positive bacilli in food smears

Culture

Bacilli may be isolated from food or faeces

Toxin Demonstration

- It can be demonstrated in food or faeces and occasionally in patient's blood or in the liver postmortem
- Toxin can also be demonstrated in food or faeces (macerated in saline and filtered—extract is used) by inoculating into mice or guinea pig intraperitoneally
- The test animal dies if toxin is present; however control—protected with polyvalent antitoxin—remains healthy

■ **Mention the treatment measures adopted for *Cl. botulinum* infections.**

- Trivalent antitoxin (A, B and E)—administered along with respiratory support
- Supportive therapy—maintenance of respiration

■ **In which year and from where was *Cl. difficile* isolated? Why is the species named as '*difficile*'?**

- *Cl. difficile* was isolated in 1935 from the faeces of newborn infants
- It was named so because it is difficult to isolate

■ **Give the characteristic morphological and cultural features of *Cl. difficile*.**

Morphological Features

- Gram-positive, long, slender bacilli
- Form large, oval and terminal spores

Cultural Features

Nonhaemolytic, saccharolytic and weakly proteolytic.

■ **Write a short note on pathogenicity of *Cl. difficile*.**

- *Cl. difficile* causes antibiotic-associated pseudomembranous colitis
- It is normally present in intestine in 3% of the general population
- Antibiotics such as ampicillin, tetracycline, chloramphenicol, lincomycin, clindamycin suppress the drug sensitive flora, allowing multiplication of *Cl. difficile* and production of enterotoxin and cytotoxin
- Enterotoxin causes diarrhoea and cytotoxin causes damage to the gut mucosa causing acute colitis with or without membrane formation

■ **What laboratory diagnostic methods should be adopted for demonstrating *Cl. difficile* infection?**

Laboratory diagnostic methods that are adopted for demonstrating *Cl. difficile* infection are:

1. Demonstration of toxin—in faeces by its characteristic effect on Hep-2 and human diploid cell cultures, or by ELISA
2. *Cl. difficile* can be isolated from faeces on selective media and tested for toxigenicity

■ **Which drugs are employed in the treatment of *Cl. difficile* infections?**

As it is associated with antibiotic treatment, current antibiotic therapy should be stopped. Metronidazole is the drug of choice. Vancomycin can also be used.

36

Chapter

Nonsporing Anaerobes

■ What are anaerobes?

Anaerobes are bacteria, which are able to live in relative or total absence of oxygen.

■ How do nonsporing anaerobes cause infection?

Nonsporing or nonclostridial anaerobes are the most common causes of anaerobic infections. The infections are always endogenous, since the nonclostridial anaerobes are obligate parasites that form the part of the normal bacterial flora of oropharynx, alimentary canal and female genital tract. These organisms from their normal habitat may invade adjacent tissues, which are debilitated or are the seat of some other pathological changes, such as reduction of tissue oxygen because of poor blood supply, tissue necrosis, and primary infection with aerobic or facultative anaerobic bacteria.

■ Name the medically important nonsporing anaerobes. Group them into cocci and bacilli.

Nonsporing anaerobes of medical importance are:

Cocci

A. Gram positive

- *Peptococcus* spp., e.g. *Pc. niger*
- *Peptostreptococcus* spp., e.g. *Pst. anaerobius*, *Pst. prevoti*, *Pst. magnus*

B. Gram negative

- *Veillonella* spp., e.g. *V. parvula*

Bacilli

A. Gram positive

- *Actinomyces* spp.
- *Arachnia* spp.
- *Bifidobacterium* spp.
- *Eubacterium* spp.
- *Lactobacillus* spp.
- *Propionibacterium* spp.

B. Gram negative

- *Bacteroides* spp., e.g. *B. fragilis*, *B. vulgates*, *B. thetaiotaomicron*, *B. distasonis*
- *Prevotella* spp., e.g. *P. melaninogenicus*, *P. denticola*, *P. buccalis*
- *Porphyromonas* spp., e.g. *P. gingivalis*, *P. endodontalis*, *P. assacharolytica*
- *Fusobacterium* spp., e.g. *F. necrophorum*, *F. nucleatum*
- *Leptotrichia* spp., e.g. *L. buccalis*

Spirochaetes

- *Treponema pallidum*
- *Borrelia* spp.

■ Give the cultural characteristics of nonsporing anaerobes.

Cultural characters of nonsporing anaerobes are:

- Being anaerobes, they grow in absence of oxygen
- Optimum temperature 37°C
- Optimum pH 7.0–7.4
- Incubation period is 48 hours or more in anaerobic conditions
- Most of them grow well on freshly prepared or prereduced blood agar
- Neomycin blood agar is used to inhibit growth of aerobes

■ Which conditions trigger development of infections by anaerobes?**Pathogenesis**

- The most important factor in developing anaerobic infections is lowering of Eh (oxidation-reduction potential), which favours multiplication of anaerobes
- Eh is lowered by—poor blood supply, tissue necrosis, growth of facultative anaerobes
- A concomitant aerobic bacterial infection may, at times, contribute synergistically in occurrence and progress of anaerobic bacterial infection
- Once the condition becomes suitable, the anaerobes invade the surrounding tissue with the help of different virulence factors, e.g. toxins, various enzymes, such as hyaluronidase, heparinase, proteases, superoxide dismutase, catalase, peroxidase, immunoglobulin proteases, which contribute to the pathogenesis
- Anaerobes also produce various extracellular products, while some anaerobes produce capsule, which prevents phagocytosis and help in pathogenesis

■ Name the diseases caused by nonsporing anaerobes.

Common infections caused by nonsporing anaerobes are:

Female Genital Tract Infections such as

- Vaginitis
- Bartholin's abscess
- Puerperal sepsis
- Post-abortion sepsis
- Pelvic abscess
- Endometritis and pyometritis
- Post-operative wound infections

Most commonly caused by *P. melaninogenicus*, *B. fragilis*, peptococci, peptostreptococci and Fusobacteria.

Abdominal Infections such as

- Appendicitis
- Diverticulitis
- Peritonitis
- Perirectal abscess
- Biliary tract infections
- Wound infections due to abdominal wound contamination after surgery or trauma

Most commonly caused by *Bacteroides* spp. and anaerobic cocci.

Ear, Nose and Throat Infections such as

- Otitis media
- Mastoiditis
- Sinusitis
- Peritonsillar infections
- Deep neck space infections

Most commonly involved anaerobes are *Fusobacteria*.

Oro-Dental Infections such as

- Gingivitis
- Dental abscess
- Mandibular space infections
- Periodontal infections
- Stomatitis

Most commonly caused by *Fusobacteria*, spirochaetes, actinomycetes and other anaerobes present in mouth.

Respiratory Infections such as

- Aspiration pneumonia
- Lung abscess
- Empyema
- Bronchiectasis

Most commonly caused by *Fusobacteria*, *P. melaninogenicus*, *B. fragilis*, anaerobic cocci and others.

Other Anaerobic Infections such as

- Bacteraemia
- Brain abscess
- Bone and joint infections
- Cellulitis
- Necrotizing fasciitis
- Ophthalmic infections
- Skin and soft tissue infections

Most commonly caused by *Bacteroides* spp., *Prevotella* spp., anaerobic cocci.

■ Describe in brief the laboratory diagnosis of anaerobic infections.**Specimens**

- Specimens should be collected carefully avoiding contamination with normal resident flora. Type of specimens collected depends on the site of infection
- Specimens collected by appropriate procedures are as follows:
 - Any closed abscess: Aspiration by needle and syringe
 - Female genital tract: Culdocentesis or aspiration
 - Lower respiratory tract: Percutaneous transtracheal aspiration or direct lung puncture
 - Pleural cavity: Thoracocentesis
 - Sinus tract and deep wounds: Syringe aspiration
 - Tissue: Aseptic surgical excision
 - Urinary tract: Needle aspiration of bladder

Transport

Transport of specimen is a crucial factor as it affects the ultimate success of the anaerobic culture. As anaerobes are extremely sensitive to oxygen, to protect them from lethal effect of oxygen from the time of collection of specimen to until they are inoculated, various techniques for safe transportation of specimen are employed.

The techniques are:

1. If a specimen is collected using a swab, it can be transported in transport media such as Stuart's medium or Robertson's cooked meat medium
2. If a specimen is collected by aspiration, immediately after collection the material is injected through a rubber stopper in a bulb containing CO₂
3. Specimen is collected in a 2 ml syringe and the needle is inserted in a sterile rubber stopper after the expulsion of air bubble
4. Blood is collected in brain heart infusion broth or thioglycollate broth for blood culture

Processing of Specimen

Direct Microscopy

- Gram-stained smear examination is important because the culture results should be compared with the Gram-stained smear results and every effort should be made to isolate all the cell morphotypes observed in the direct smear of the specimen
- Gram stain usually shows polymicrobial picture, i.e. it consists of many types of bacteria

Culture

Specimen is inoculated on selective and nonselective media, to ensure the culture of all anaerobes present in the specimen. The commonly recommended media for primary culture of anaerobes are:

- Plain freshly prepared blood agar—for all anaerobes
- Neomycin blood agar—for all anaerobes
- Neomycin—kanamycin blood agar—for all anaerobes
- Phenyl—ethyl alcohol blood agar—for all anaerobes
- Kanamycin—vancomycin blood agar—for Gram-negative anaerobes

After inoculation, plates are incubated anaerobically in McIntosh-Filde's jar or gaspak jar.

Identification

The colony morphology of each different type of colony is noted. Each type of colony is Gram stained and subcultured for oxygen tolerance by inoculating on blood agar and incubating aerobically.

The oxygen tolerant colony is considered as anaerobe and the identification scheme is continued. The identification of anaerobes can often be made on the basis of:

- Colony morphology
- Pigmentation
- Haemolysis
- Pitting of agar
- Fluorescence under ultraviolet light
- Motility
- Catalase test
- Indole test
- Sugar fermentation

- Bile resistance
- Esculin hydrolysis

■ **Describe the rapid identification methods for detection of nonsporing anaerobes.**

Rapid Identification Methods

Rapid Identification with the Help of Gentamicin and Metronidazole Disc

The rapid presumptive identification of anaerobes can be made by using discs of gentamicin (10 µg) and metronidazole (5 µg), applied on heavily inoculated plate at the primary and secondary streaking, respectively. Majority of anaerobes are susceptible to metronidazole and resistant to gentamicin. The culture plates after incubation are observed for zone of inhibition surrounding the metronidazole disc and a representative colony at the margin of the zone is tested for aerobic growth. A colony failing to grow aerobically from such a margin is identified as an obligate anaerobe.

Rapid Identification by Biochemical Tests

Various rapid microtechniques have been developed. These include:

1. The API—20 A strip containing carbohydrates and tests for indole, urease, gelatin, esculin and catalase
2. The minitek (BBL) system

■ **Describe the immunological methods, which aid in the diagnosis of nonsporing anaerobic infections.**

Various immunological methods that can be used for detection of antibodies are:

- Indirect immunofluorescence test
- Indirect haemagglutination test
- Counter current immunoelectrophoresis (CIEP)
- Enzyme-linked immunosorbent assay (ELISA)
- Microagglutination test
- Complement fixation test (CFT)

■ **Describe the different tests used for detection of antigens.**

Methods used for detection of antigens are:

1. Co-agglutination test
2. Direct immunofluorescence test
3. Agglutination test
4. Haemagglutination test
5. Polyacrylamide gel electrophoresis

■ **Comment on the antibiotic susceptibility testing of nonsporing anaerobes.**

- Susceptibility of different anaerobic species to various antimicrobial agents is moderately constant and predictable. Hence, routine susceptibility testing is unnecessary and may be conveniently replaced by a system of periodic monitoring
- Routine susceptibility testing is generally recommended, when determination of the susceptibility of individual isolate is of great importance, e.g. serious infections, such as endocarditis or brain abscess and infections that fail to respond to empirically selected therapy
- Methods used for susceptibility testing are:
 1. Broth dilution method
 2. Agar dilution method

3. Broth disc test
4. Disc diffusion method—commonly used

■ **Name the antibiotics commonly prescribed for treating infections caused by nonsporing anaerobes.**

Antibiotics commonly used in the treatment of infections caused by nonsporing anaerobes are:

- Penicillin
- Tetracycline
- Chloramphenicol
- Clindamycin—not commonly recommended
- Metronidazole—drug of choice
- Fucidin
- Trimethoprim
- Some infections require surgical intervention also
- Metronidazole can also be used as a prophylactic agent in large bowel surgery

37

Chapter

Enterobacteriaceae I: *Escherichia coli*, *Klebsiella* and *Proteus*

■ Describe briefly the general features of Enterobacteriaceae.

- The members of the family Enterobacteriaceae, consists a large number of closely related bacterial species that inhabit large intestine of man and animals, soil, water and decaying organic matter; also referred as enteric bacteria
- They are aerobic or facultative anaerobic Gram-negative bacilli, motile by peritrichate flagella or are nonmotile, nonsporing and noncapsulated
- They grow on ordinary media, ferment glucose with acid or acid and gas, oxidase negative and catalase positive, except *Shigella dysenteriae*
- The family includes some of the most important intestinal pathogens for humans, e.g. *Salmonella* and *Shigella*
- Many members do not cause disease when they are in gut, but when their habitat is changed they become pathogenic
- They are Gram-negative bacilli, classified based upon lactose fermentation as

1. Lactose fermenters	2. Nonlactose fermenters	3. Late lactose fermenters
Ferment lactose, produce pink colonies on MacConkey's agar, e.g. <i>E. coli</i> , <i>Klebsiella</i>	Do not ferment lactose, produce colourless colonies on MacConkey's agar, e.g. <i>Salmonella</i> , <i>Shigella</i> , <i>Proteus</i>	Ferment lactose late, hence initially colourless colonies, which become pink after prolonged incubation, e.g. <i>Shigella sonnei</i>

■ Name the scientist who first described *Escherichia*.

The genus *Escherichia* is named after the scientist, Escherich, who first described the bacillus in 1881. *E. coli* is the medically important species.

■ State the characteristic morphological features of *Escherichia coli*.

Morphological Features

- Gram-negative bacillus
- Size: $1-3 \mu \times 0.4-0.7 \mu$
- Motile by peritrichous flagella
- Possess fimbriae
- Nonsporing and noncapsulated (few strains especially isolated from extra-intestinal sites are capsulated)

■ Mention the cultural characteristics of *E. coli*; include media and colony characters.

Cultural Characters

- Aerobe and facultative anaerobe
- Grow at a wide range of temperature 10–40°C, optimum 37°C
- Grow on ordinary media like nutrient agar

Media and Colony Characters

- **Nutrient agar:** Large, 2–3 mm in diameter, circular, low convex, opaque, gray-white colonies
- **Blood agar:** Beta-haemolytic colonies
- **MacConkey's agar:** Pink flat (low convex) colonies—due to lactose fermentation (Fig. 37.1)

■ Which biochemical reactions help in the identification of *E. coli*?

- **Sugar fermentation reactions**—ferments glucose (G), lactose (L), sucrose (S) and mannitol (M) with acid and gas
- **IMViC test**—indole (I) and methyl red (MR) tests are positive, and Voges-Proskauer (VP) and citrate (C) are negative
- **TSI**—(triple sugar iron)–A/A (A-acid)
- **Urease and H₂S** —negative

■ Mention the resistance of *E. coli*.

- *E. coli* can survive for several days in soil and water
- It is killed at 60°C within 30 minutes
- Chlorination of water kills *E. coli*

■ Name the antigens that are present in *E. coli*.

Four types of antigens are present. These are:

1. H Ag—It is a flagellar Ag-75 H Ags
2. O Ag—It is a somatic Ag-173 O Ag



Fig. 37.1 *E. coli*: Lactose fermenting moist colonies on MacConkey's agar.

3. K Ag—It is a capsular Ag-103 Ags
4. F Ag—It is a fimbrial Ag

■ How is antigenic typing done in *E. coli*?

Antigenic typing in *E. coli* is done on the basis of O Ags:

- *E. coli* is divided into O groups
- Each O group is divided then into subgroup on the basis of K Ag
- Each subgroup includes strains with different H Ag
- So a strain of *E. coli* is designated, e.g. as O₁₁₁: K₅₈: H₂

■ Describe the different types of toxins produced by *E. coli*.

Toxins produced by *E. coli* are:

- Enterotoxin
- Haemolysins
- Verotoxin

Enterotoxin

- *E. coli* produces two different enterotoxins. These are:
 - Heat labile toxin (LT)
 - Heat stable toxin (ST)
- Its production is genetically controlled by a plasmid

Heat labile toxin (LT)

- It is destroyed by heating—heat labile
- **Mechanism of action:** It has 2 subunits, A and B. Subunit B binds to Gm ganglioside receptors on epithelial cells of small intestine and subunit A is released. Subunit A then enters the cell and catalyses NAD-dependent action of membrane bound adenylate cyclase leading to conversion of ATP to cAMP, which inhibits absorption of Na, Cl and water in the cells and increases Na secretion and causes loss of chlorides and water.

The resultant water and electrolytes outflow into the lumen of the small intestine, which results in profuse watery diarrhoea.

Heat stable toxin (ST)

- Heating does not destroy it
- **Mechanism of action:** It acts by activation of guanylate cyclase. It leads to formation of cGMP in epithelial cells, which causes inhibition of co-transport of sodium chloride into wall, i.e. anti-absorptive action resulting in diarrhoea

Methods of Demonstration of Enterotoxins

They can be demonstrated by using various tests (Table 37.1).

Haemolysins

- Cause lysis of red blood cells
- Strains isolated especially those from extra-intestinal specimens are haemolytic

Vero Cytotoxin

- It is like *Shigella* toxin, two types—VT1 and VT2
- Cytotoxic to both Vero and HeLa cells

Table 37.1 Methods to demonstrate enterotoxin of *Escherichia coli*

Test	Heat labile toxin	Heat stable toxin
1. In vivo tests		
– Ligated ileal loop test		
■ Read at 6 hours	– or +	+
■ At 18 hours	+	–
– Infant mouse intragastric at 4 hours	–	+
– Adult rabbit skin (vascular permeability factor)	+	–
2. In vitro tests		
– Tissue culture tests		
■ Rounding of Y1 mouse adrenal cells	+	–
■ Elongation of Chinese hamster ovary (CHO) cells	+	–
3. Serological tests		
– ELISA	+	–
– PHA	+	–
4. Genetic test		
– DNA probe	+	–

ELISA = enzyme-linked immunosorbent assay, PHA = passive haemagglutination, + = positive, – = negative.

■ Describe in detail the pathogenicity of *E. coli* infection.

E. coli can cause four main types of infections. These are:

1. Urinary tract infections
2. Diarrhoea
3. Pyogenic infections
4. Septicaemia

Urinary Tract Infections

- *E. coli* accounts for large number of naturally acquired urinary tract infections
- They usually cause ascending urinary tract infections, while strains carrying K Ag are responsible for descending (haematogenous) urinary tract infections

Diarrhoea and Dysentery

Five different types of *E. coli* are responsible for causing diarrhoea and dysentery. These are:

1. Enteropathogenic *E. coli* (EPEC)
2. Enterotoxigenic *E. coli* (ETEC)
3. Enteroinvasive *E. coli* (EIEC)
4. Enterohaemorrhagic *E. coli* (EHEC)
5. Enteroaggregative *E. coli* (EAEC)

Enteropathogenic *E. coli* (EPEC)

- They are usually associated with diarrhoea in infants and children and less often in adults
- They are responsible for outbreaks as well as sporadic cases
- **Mechanism of action:** attach to mucosa of small intestine causing disruption of brush border of microvilli

- **Identification:** can be identified by their adhesion to HEP-2 cells and colony agglutination by polyvalent and monovalent EPEC O antisera

Enterotoxigenic E. coli (ETEC)

- ETEC is endemic in tropics, and causes diseases that range in severity from mild diarrhoea to cholera-like disease
- They also cause traveller's diarrhoea
- **Mechanism of action:** Adhesion of bacteria and production of heat labile and heat stable toxins
- **Demonstration:** Toxin production can be demonstrated by various tests mentioned in Table 37.1

Enteroinvasive E. coli (EIEC)

- They are invasive, cause diseases like shigellosis
- They are nonmotile and nonlactose fermenters
- **Mechanism of action:** Invasiveness, which is determined by plasmid, codes for outer membrane antigen called virulence marker antigen (VMA)
- **Demonstration:**
 - (i) Penetrate HeLa cells and HEP2 cells in culture
 - (ii) Sereny test—Instillation of isolate into eyes of guinea pig or mice produces conjunctivitis and keratitis
 - (iii) VMA—Virulence marker Ag detection by ELISA

Enterohaemorrhagic E. coli (EHEC)

- Also called Verotoxigenic *E. coli* (VTEC), causes mild diarrhoea to fatal hemorrhagic colitis and hemorrhagic uraemic syndrome in children and adults
- **Mechanism of action:** Action is mediated by Verotoxin, which acts on vascular endothelial cells
- **Demonstration:**
 - Demonstration of Verotoxin in culture of faeces by DNA probes for VT1 and VT2 genes
 - Cytotoxic effect on Vero and HeLa cells
 - Demonstration of Verotoxin neutralizing antibody in sera

Enteroaggregative E. coli (EAEC)

- They are named so because they appear aggregated in stacked-brick formation
- They are associated with persistent diarrhoea in developing countries
- **Mechanism of action:** It is mediated by enteroaggregative heat stable enterotoxin-1

Pyogenic Infections

- Wound infections
- Peritonitis
- Biliary tract infections
- Main cause of meningitis in newborn

Septicaemia

- *E. coli* is a common cause of septicaemia in many hospitals, usually occurs in debilitated patients and associated with high mortality.
- Clinically it is manifested as fever, hypotension and disseminated intravascular coagulation

- Describe the procedures adopted and examinations conducted in laboratory diagnosis of (a) Urinary tract infections, (b) Diarrhoea, and (c) Pyogenic infections and septicaemia

A. Urinary Tract Infections

Specimen

Urine

Collection

- Clean voided midstream urine sample is ideal for culture, which should be collected after local cleaning in a wide mouthed sterile, dry, leak-proof container
- It can be collected by catheterization
- In infants it can be aspirated by suprapubic puncture from bladder with syringe and needle

Transport

- Urine should reach the laboratory within 1 hour of collection
- If delay is unavoidable, sample should be refrigerated at 4°C
- If more delay is expected, 0.1 g/10 ml of boric acid powder is added to preserve specimen

Microscopic Examination

- **Wet preparation:** Wet preparation is examined for presence of bacteria, pus cells, red blood cells, crystal, cast and epithelial cells
- Presence of 3 pus cells/high power field (HPF) suggests infection
- **Gram stain:** Sediment after centrifuging urine is used for preparation of smear, it shows presence of Gram-negative bacilli

Culture

- For routine culture semi-quantitative "Standard loop" technique is used in which measured quantity (0.004 ml or 1/250 ml) of urine is inoculated with standard loop made up of nichrome or platinum wire on blood agar (BA) and another loopful on MacConkey's agar (BA is helpful for quantitative measurement of bacteriuria)
 - After overnight incubation at 37°C, colonies on BA are counted and multiplied by 250 to get bacterial count per millilitre
 - On the basis of the results, culture can be reported as significant or insignificant bacteriuria, counts more than 100,000/ml of urine are considered as significant and counts less than 100,000/ml should be reported as insignificant bacteriuria.
- Other semi-quantitative techniques are:
 - Filler paper strip
 - Dip spoon
 - Dip slide

Colony

- **Blood agar:** Large, circular, gray-white, opaque, beta-haemolytic colonies
- **MacConkey's agar:** Lactose fermenting flat colonies (pink)

Biochemical Reactions

L	G	M	S	I	M	Vi	C	TSI	Urease	H ₂ S
+	+	+	-	+	+	-	-	A/A	-	-

(+= positive, - = negative).

Antibiotic Sensitivity Testing

- Kirby–Bauer disc diffusion method is used.
- Isolate tested using nitrofurantoin, nalidixic acid, ampicillin, gentamicin, amikacin, amoxycillin, quinolones, cotrimoxazole, first, second and third generation cephalosporins, etc.

B. Diarrhoea**Specimen**

Faeces or rectal swab.

Collection

- Stool is collected in bedpan and portion of it containing mucus flakes is transferred to transport media
- Swab from ulcer is collected by sigmoidoscopic examination

Transport of Specimen

Specimen is transported in transport medium to microbiology laboratory.

Culture

Specimen is inoculated on

- **Blood agar:** Large, 2–3 mm in diameter, circular, low convex, opaque, gray white, beta-haemolytic colonies
- **MacConkey's agar:** Pink flat (low convex) colonies—due to lactose fermentation

The typical colonies are identified using standard biochemical reactions

Serology

EPEC—colonies emulsified in saline and tested by polyvalent and monovalent EPEC O antisera for identification.

Identification of Other Types *E. coli*

EIEC, ETEC, EHEC by appropriate tests.

C. Pyogenic Infection and Septicaemia

- Pus or swab from wound in pyogenic infection is processed as above
- Blood in septicaemia is processed for blood culture in a routine way

■ Comment in short on treatment of *E. coli* infections.

Treatment of *E. coli* infections should always be guided by antibiotic sensitivity testing because of problem of multiple drug resistance in *E. coli* and ESBL producing strains.

■ Write the important features of (a) *Enterobacter*, (b) *Serratia*, (c) *Citrobacter*.**(a) *Enterobacter***

- Gram-negative motile bacilli
- Two species:
 1. *E. cloacae*
 2. *E. aerogenes*
- They are indole and MR negative and VP citrate positive. They are involved in
 - Urinary tract infections (UTI)
 - Sepsis

(b) *Serratia*

- Gram-negative bacilli
- Species: *S. rubidaea*, *S. marcescens*, and *S. liquefaciens*
- Motile rods—some strains produce—nondiffusible red pigment—prodigiosin
- Ferments lactose—slowly
- Causes infection in hospitalized patients. These include:
 - Urinary tract infections
 - Respiratory tract infections
 - Meningitis
 - Wound infections
 - Septicaemia
 - Endocarditis
 - Endotoxic shock
 - Resistant to cephalosporins

(c) *Citrobacter*

- Gram-negative motile bacilli
- Grows on blood agar and MacConkey's agar- late lactose fermenter
- I MR VP C H₂S L G S
V + - + + (+) + V
(+ = positive, - = negative, V = variable, (+) = late fermenter)
- Important species—*Citro. freundii*, *Citro. koseri*, *Citro. amalonaticus*
- Ag-sharing with *Salmonella*—cause confusion in diagnosis
- Some strains possess 'Vi' Ag identical to *S. typhi* and *S. paratyphi C*
- Normally present in intestine
- Cause infections of
 - Urinary tract
 - Gallbladder
 - Middle ear
 - Meningitis

■ Name some medically important species of *Klebsiella*.

Medically important species of *Klebsiella* are:

- *K. pneumonia*
- *K. oxytoca*
- *K. planticola*
- *K. ornithinolytica*

■ Write the names of subspecies of *Klebsiella pneumoniae*.

Subspecies of *K. pneumoniae* are:

- *K. ozaenae*
- *K. pneumoniae*
- *K. rhinoscleromatis*
- *K. aerogens*

■ State the characteristic morphological features of *Klebsiella*.**Morphological Features**

- Gram-negative bacilli
- Size about 1–2 μ \times 0.5–0.8 μ

- Sides parallel or bulging, slightly pointed or rounded ends, singly or in pairs
- Nonmotile and nonsporing
- Most strains possess fimbriae
- Capsulated- can be demonstrated by
 - **Quellung reaction**—appears swollen, sharply delineated and refractile
 - **Gram's stain**—appears as unstained halo around bacteria
 - **India-ink**—appears as unstained area around bacteria with blue background

■ Give the cultural characteristics of *Klebsiella*; include media and colony characters.

Cultural Characters

- Aerobe and facultative anaerobe
- Can grow on ordinary media-like nutrient agar
- Optimum temperature 37°C and optimum pH 7.2–7.4

Media and Colony Characters

Nutrient agar: Large, mucoid, grayish-white, smooth with entire margins

MacConkey's: LFM colonies (lactose-fermenting mucoid) Fig. 37.2.

■ Which biochemical reactions are characteristic of *Klebsiella*?

Biochemical Reactions

- | | | | | | | | | |
|---|---|---|---|---|---|---|----|---|
| • | L | G | M | S | I | M | Vi | C |
| | + | + | + | + | – | – | + | + |

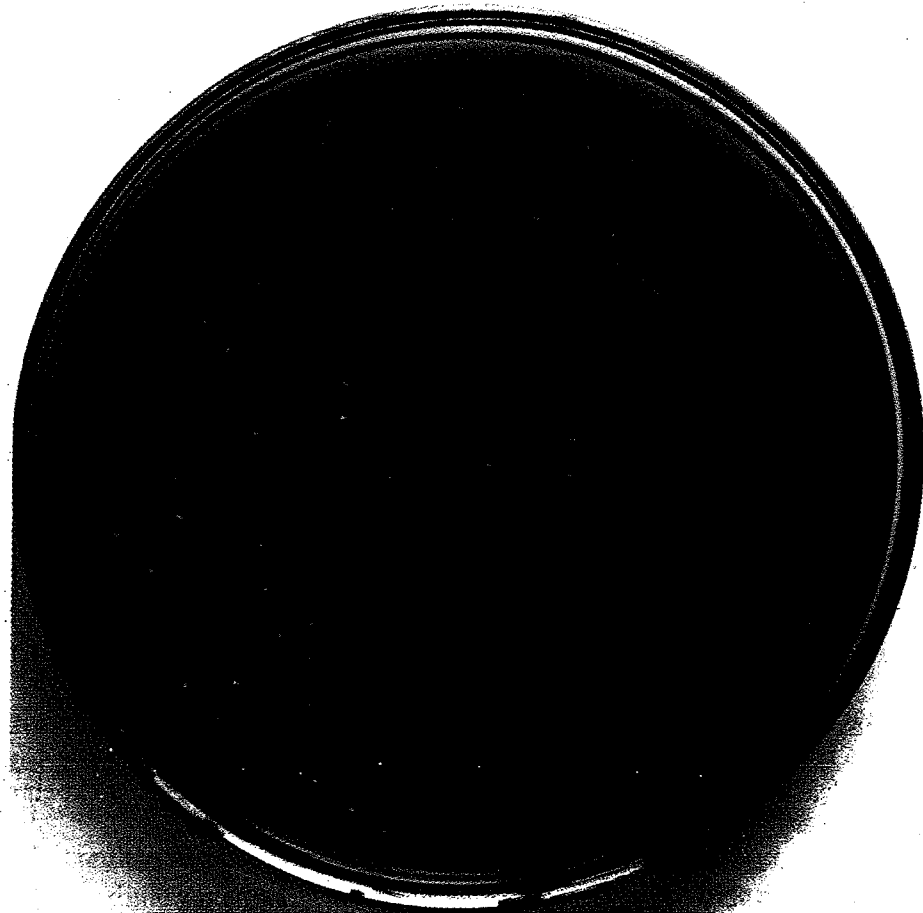


Fig. 37.2 *Klebsiella* spp.: Lactose fermenting mucoid colonies on MacConkey's agar.

- Urease H_2S TSI (triple sugar iron)
+ - A/A with gas
(+ = positive, - = negative, A = acid)
- *K. pneumoniae*—indole-negative
- *K. oxytoca*—indole-positive

■ Comment on antigens present in *Klebsiella*.

Antigens present in *Klebsiella* and their location are:

- K Ag—capsular antigen
- O Ag—somatic antigen masked by K antigen
- On the basis of capsular K Ag, there are 1–80 serotypes of *Klebsiella*

■ Which two important species of *Klebsiella* are pathogenic to human beings?

Two important species of *Klebsiella*, which are pathogenic to humans are:

1. *K. pneumoniae*
2. *K. oxytoca*

■ Name the infections caused by *Klebsiella*.

Various infections caused by *Klebsiella* are:

1. Respiratory infections—Bronchopneumonia, pleuritis, multiple abscesses in lung
2. Hospital acquired urinary tract infection
3. Wound and burn infections
4. Bacteraemia
5. Atrophic rhinitis with ozaena—caused by *K. ozaenae*
6. Rhinoscleroma—chronic upper respiratory tract disease with lesion in nose, larynx and throat—caused by *K. pneumoniae rhinoscleromatis*
7. Nosocomial infections
8. Pyogenic infections such as abscesses, meningitis

■ Discuss in brief the laboratory diagnosis of *Klebsiella* infection.

Specimens

Sputum, urine, wound or burns swab, pus and cerebrospinal fluid

Collection

- Sputum—In a wide-mouthed sterile container
- Urine—Midstream urine sample in a sterile plain bulb or culture tube
- Pus—Aspirated or collected with cotton swab
- Cerebrospinal fluid—with all aseptic precautions by lumbar puncture collected in a sterile container

Transport

- Cerebrospinal fluid should reach as early as possible to laboratory
- Stuart's media is used for swab
- Urine should reach the laboratory within 1 hour of collection; if delay is expected it should be refrigerated

Microscopic Examination

- Gram stain—shows capsulated, Gram-negative bacilli

Culture

Sample is inoculated on blood agar and MacConkey's agar aerobically. MacConkey's agar showing lactose-fermenting mucoid colonies are further identified by biochemical reactions

Biochemical Reactions

- Ferments LGMS with acid and gas
- Indole and MR—Negative
- VP and citrate—Positive
- Urease—Positive
- H₂S—Negative –
 - *K. pneumoniae*—indole-negative
 - *K. oxytoca*—indole-positive

Antibiotic Sensitivity Testing (AST)

- By Kirby–Bauer disc diffusion method
- Isolate is tested using first, second and third generation cephalosporins, amikacin, gentamicin, co-amoxiclav, fluoroquinolones, co-trimoxazole, etc. Nitrofurantoin is used for urinary isolates

■ **Mention the three genera belonging to the tribe Proteae.**

Tribe Proteae consists of following three genera:

1. *Proteus*
2. *Morganella*
3. *Providencia*

■ **Name some important species of *Proteus*.**

Important species of *Proteus* are:

- *P. vulgaris*
- *P. mirabilis*
- *P. myxofaciens*
- *P. penneri*

■ **State the salient morphological features of *Proteus*.****Morphological Features**

- Gram-negative coccobacilli
- Pleomorphic (named *Proteus* after the Greek God *Proteus* who could assume any shape)
- Size: 1–3 $\mu \times$ 0.6 μ ; long filaments up to 80 μ are also seen
- Actively motile by peritrichous flagella and possess fimbriae
- Nonsporing and noncapsulated

■ **Give the cultural characteristics of *Proteus*; include media and colony characters.****Cultural Characters**

- Aerobe and facultative anaerobe
- Can grow on ordinary media like nutrient agar
- Culture has seminal odour or fishy odour

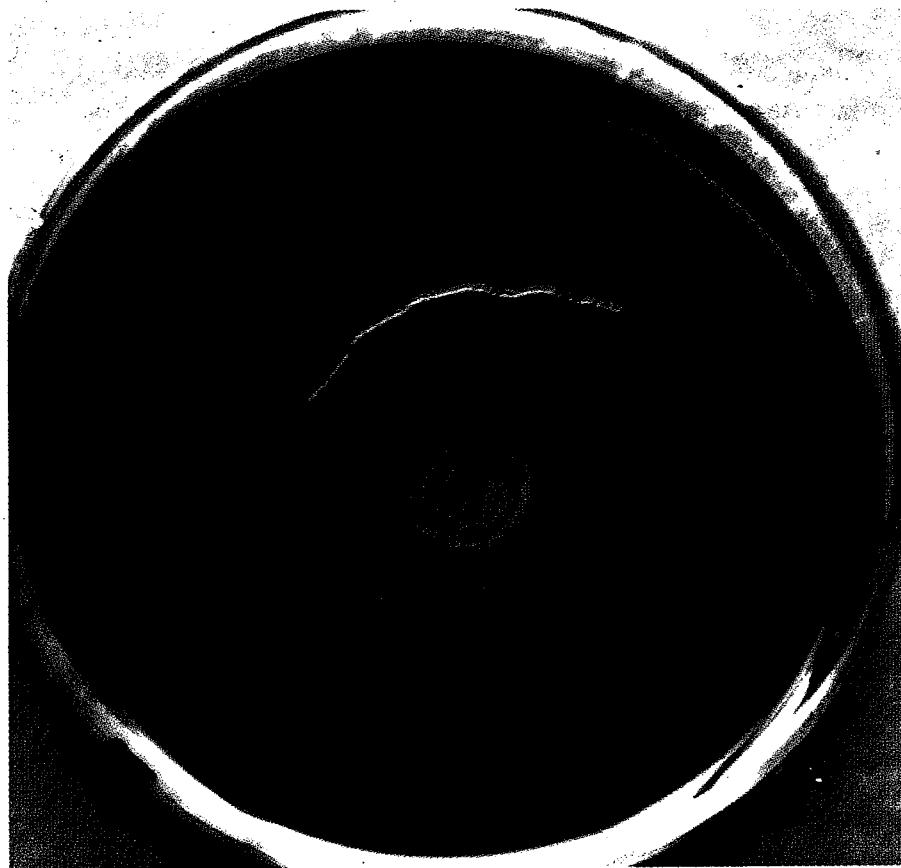


Fig. 37.3 *Proteus*: Swarming growth on blood agar.

Media and Colony Characters

- **Blood agar:** *Proteus* shows swarming. This is a characteristic feature of *Proteus*—a group of cells at the edge of developing colony migrate to un-inoculated area of the medium, to form uniform film of growth extending over the whole plate (Fig. 37.3)
- **MacConkey's agar:** Colourless colonies because of nonlactose fermentation

■ Name and define different types of swarming.

Swarming is of two types:

1. Continuous
2. Discontinuous
 - **Continuous swarming:** Swarming in which uniform film of growth is formed on plate is called continuous swarming
 - **Discontinuous swarming:** Swarming in which series of concentric circles of growth around point of inoculation are formed is called discontinuous swarming

■ What are the possible causes of swarming? How can it be inhibited?

Possible Causes of Swarming

- Positive chemotactic response to deteriorating nutritional condition
- Negative chemotactic response to metabolic products that are accumulated in the agar, in areas of high population density

- Swarm cell formation arises under condition of rapid growth, in which, as a result of depletion of nutrients, flagellar synthesis becomes uncontrolled and this leads to deficiency of substances necessary for growth

Antiswarming Agents

Swarming of *Proteus* makes it difficult to isolate the other bacterial pathogen in pure culture with which it is present in clinical specimen.

Ways of Inhibiting Swarming

- Physically restricting movement of *Proteus* cells by
 - Agar overlays
 - Poured plates
 - By increasing agar concentration to 3–4%
- Preventing the formation of or interfering with structure or activity of flagella by
 - Incorporation of H antisera
 - Ethanol (5.5%)
 - Boric acid (0.1%)
 - Detergents
 - Bile salt
- Retarding growth rate by incorporation of
 - Sulphonamides
 - Neomycin
 - Chloral hydrate
 - Barbiturate
 - Sodium azide
- Unexplained mechanism
 - Activated charcoal
 - P-nitro-phenyl-glycerol
 - Swarming is also inhibited on MacConkey's agar—because of bile salts
 - Wilson and Blair media—because of Bismuth sulphite
 - Cysteine lactose electrolyte deficient media (CLED)—because of absence of electrolytes

■ Mention the biochemical reactions characteristic of *Proteus*.

- The characteristic biochemical reactions are:

L	G	M	S	I	M	Vi	C	H ₂ S	Urease	PPA	TSI (Triple sugar iron)
–	+	–	–	V	+	–	V	+	+	+	K/A with H ₂ S

 (+ = positive, – = negative, V = variable, K = alkaline, A = acid)
- *Pr. mirabilis* and *Pr. vulgaris* are common isolates and are differentiated by indole test; they are negative and positive, respectively

■ Explain Diene's phenomenon.

When two identical strains of *Proteus* are inoculated at different points of the same culture plate, without any swarming-inhibiting substance, the resultant swarming of growth coalesce without signs of demarcation. However, when two different strains of *Proteus* species are inoculated, the spreading films of growth fail to coalesce and remain separated by narrow but easily visible furrow. This is known as Diene's phenomenon.

Use: It is used to determine the identity or nonidentity of various strains of *Proteus*.

■ Mention the antigens possessed by motile and nonmotile strains of *Proteus*.**Antigens Possessed by Motile Strains**

- O—somatic
- H—flagellar Ag

Weil and Felix while studying *Proteus* observed that flagellated strains growing on agar formed a thin surface film resembling the mist produced by breathing on glass and named this variety as Hauch (meaning film of breath). Nonflagellated variants grew as isolated colony without surface film and were called Ohne Hauch (meaning without film of breath). These two names were then abbreviated as H and O forms.

Antigens Possessed by Nonmotile Strains

Weil and Felix also observed that certain nonmotile strains of *Pr. vulgaris* called 'X strains' were agglutinated by sera of typhus fever patient. This is heterophile agglutination due to sharing of alkali-stable fraction of O Ag of OX2, OX K and OX19 by certain Rickettsial strains (O Ag has got alkali-labile and alkali-stable fractions). This is the basis of Weil-Felix reaction, used for diagnosis of some Rickettsial diseases. OX19, OX2 are nonmotile strains of *Pr. vulgaris* and OX K is of *Pr. mirabilis*.

■ Describe the diseases caused by *Proteus* spp.

Proteus causes the following diseases:

Urinary Tract Infections (UTI)

- Urinary tract infections caused by *Proteus* tend to be more serious than that by *E. coli* and other coliforms because:
 - *Proteus* has a predilection to upper urinary tract
 - It produces Urease enzyme, which liberates ammonia from urea, ammonia inactivates complement, damages renal epithelium and makes urine alkaline
- Alkaline pH of urine due to production of ammonia leads to
 - Precipitation of phosphates and formation of calculi
 - Possibly hyperammonaemic encephalopathy and coma

Pyogenic Infections

- Wound infections, bedsores, osteomyelitis and infection of umbilical stump leading to meningitis or bacteraemia
- It can also cause ear and respiratory tract infections

■ Describe the laboratory diagnosis of *Proteus* infections.**Specimens**

Urine, pus, ear swab, wound swab, cerebrospinal fluid and sputum.

Collection

- Urine: Fresh midstream urine sample in a sterile container
- Pus: Aspirated with syringe and collected in a sterile plain bulb
- Ear swab/wound swab: Cotton swab is used to collect material
- Cerebrospinal fluid: With all aseptic precautions lumbar puncture is done and collected in a sterile container
- Sputum: Early morning sample in dry, wide-mouthed, leakproof container

Transport

Urine—should be refrigerated immediately if there is delay in transport.

Microscopic Examination

- **Wet preparation** (Urine, CSF)—plenty of pus cells with rods
- **Gram Stain**—Gram-negative pleomorphic rods with pus cells

Culture

- Blood agar—swarming
- MacConkey's agar—nonlactose fermenting colonies

Biochemical Reactions

- Catalase—positive
- Oxidase—negative
- L G M S I M Vi C H₂S Urease PPA
 – + – – V + – V + + +
 (+ = positive, – = negative, V = variable)

Pr. mirabilis and *Pr. vulgaris* are common isolates and are differentiated by indole test, they are negative and positive respectively.

Antibiotic Sensitivity Testing

- Kirby–Bauer disc diffusion method is used
- Isolate tested for various antibiotics – gentamicin, amikacin, cotrimoxazole, fluoroquinolones and cephalosporins
- Usually they are resistant to polymyxin B and colistin
- Sensitive to ampicillin, cefotaxime, cefuroxime, aminoglycosides
- The strains are known to produce beta-lactamase

■ **Write a short note on *Providencia*.*****Providencia***

- Genus has five species. These are:
 1. *Pro. Rettgeri*
 2. *Pro. alcalifaciens*
 3. *Pro. Stuartii*
 4. *Pro. rustigianii*
 5. *Pro. heimbachae*
- All are motile but do not swarm
- Colony has fruity smell
- **Pathogenicity**—urinary tract infections, pneumonia, wound infections and hospital acquired infection
- Laboratory diagnosis is done by same steps as in *Proteus*

Enterobacteriaceae II: *Salmonella*

■ **How is the genus *Salmonella* medically significant? Mention the names of scientists who have contributed in this?**

- Genus *Salmonella* consists of Gram-negative bacilli that parasitize the intestinal tract of large number of vertebrates and infect human beings
- Three major diseases caused by them are:
 1. Enteric fever
 2. Gastroenteritis
 3. Septicaemia
- Genus has been named after the microbiologist Salmon
- It consists of about 2300 serotypes as described by Kauffman–White scheme
- The most important member of the genus *Salmonella* is *Salmonella typhi*—the causative agent of typhoid fever
- It was first observed by Eberth (1880) and was isolated by Gaffky (1884), so it was also called Eberth–Gaffky bacillus
- Other members are *S. paratyphi* A, *S. paratyphi* B, and *S. paratyphi* C, *S. typhimurium*, *S. choleraesuis*, *S. enteritidis*, *S. gallinarum-pullorum* and *S. anatum*

■ **State the distinguishing morphological features of *Salmonella*.**

Morphological Features

- Gram-negative bacilli
- Size: About $1-4 \mu \times 0.6 \mu$
- Motile with peritrichous flagella (exception *S. gallinarum-pullorum*)
- Noncapsulated and nonsporing
- May possess fimbriae

■ **Mention the cultural characteristics of *Salmonella*, including media and colony characters.**

Cultural Characters

- Aerobes and facultative anaerobes
- Can grow on ordinary media-like nutrient agar
- Optimum temperature 37°C (range, $15^{\circ}-41^{\circ}\text{C}$) and optimum pH 7.2–7.4 (range, 6–8)

Media and Colony Characters

- **Blood agar and nutrient agar:** Large, 2–3 mm in diameter, circular, convex, grayish-white and translucent colonies
- **MacConkey's agar and deoxycholate citrate agar:** Colourless colonies because of nonlactose fermentation (Fig.38.1a)



Fig. 38.1a Nonlactose fermenting colonies on MacConkey's agar.

- **Selective Medium:** Wilson and Blair medium
 - Jet black colonies with metallic sheen by H_2S -producing *Salmonellae* (Fig. 38.1b)
 - Green by the species, which do not produce H_2S
- **Enrichment media**
 - Selenite F broth
 - Tetrathionate broth
 - Gram-negative broth
- **Biphasic culture media** (Castaneda's medium; Fig. 44.1)
 - As blood and bone marrow culture has to be examined for 10 days before declaring negative, there is a risk of contamination with every subculture
 - To eliminate risk of introducing contamination during subcultures and also for safety and economy, biphasic culture media are used
 - Biphasic medium consists of a bottle with agar slant along with broth
 - Blood is inoculated in broth, bottle is incubated in upright position and for subculture, bottle is tilted so that broth runs over the slant and then bottle is incubated in upright position
 - Next day slant is observed for presence of colonies

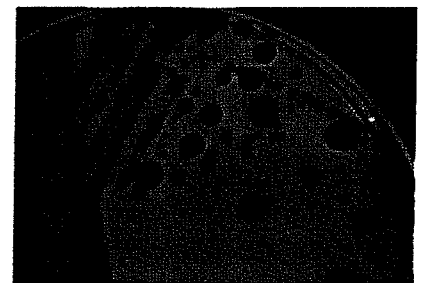


Fig. 38.1b Jet black colonies on Wilson and Blair medium. (Source: *Journal of Linné and Ringsrud's Clinical Laboratory Science: Introduction to Microbiology*. Figure 16-13, 2007, Elsevier.)

■ **Mention the biochemical reactions that are characteristic of *Salmonella* spp.**

Biochemical reactions characteristic of *Salmonella* spp. are:

- L G M S — all species ferment G, M with acid and gas except *S. typhi*
– + + – (anaerogenic ferments without gas)
- I M Vi C — all are citrate-positive except *S. typhi* and *S. paratyphi* A
– + – +
- PPA and urease—negative
- H_2S —positive, except *S. paratyphi* A and *S. choleraesuis*
- TSI (K/A) with H_2S (K—alkaline, A—acid)

■ **In what ways can *Salmonella* be inactivated and killed?**

- *Salmonella* can be inactivated by heat at 60°C in 15 minutes
- It can be killed by boiling, chlorination and pasteurization

■ **Name the antigens present in *Salmonella*.**

- Four antigens are present in *Salmonella*. These are:
 - H—Flagellar antigen
 - O—Somatic antigen
 - Vi—Surface antigen
 - F—Fimbrial antigen
- Identification and consequently classification of *Salmonella* on the basis of antigens is based on H, O and Vi Ag
- F Ags is not useful in identification because of their nonspecific nature and widespread sharing among enterobacteria.

■ **Write a short note on each of the following antigens of *Salmonella*: H antigen, O antigen, and Vi antigen.**

Flagellar or H Ag

- It is heat-labile flagellar antigen
- When mixed with its antisera, H antigen agglutinates rapidly by producing large, loose, fluffy clumps
- H is more immunogenic than O antigen, so induces antibody formation rapidly and in high titres following infection or immunization
- It is dual in nature, occurring in one of the two phases:
 - **Phase I antigen:** It is either specific for serotype or shared by few serotypes only. Hence, phase I is specific phase
 - **Phase II antigen:** These are widely shared and hence, it is a nonspecific phase
- Large numbers of flagellar antigen have been found in phase I. These are designated by small letters of alphabet—a to z except j and then as z1 to z68
- In phase II—antigens are designated by Arabic numerals (1–12)
- Some strains that possess both phases are known as diphasic and strains that possess only one phase are known as monophasic

Somatic (O) Antigen

- It is heat-stable phospholipid–protein–polysaccharide complex, which forms an integral part of cell wall
- When mixed with antisera, O antigen forms compact, chalky, granular clumps
- O agglutination takes place slowly and at higher temperatures (50°–55°C)
- O antigen is less immunogenic than H antigen and titre of the O antibody induced after infection or immunization is generally lower than that of H antibody
- O antigen is not a single factor but a mosaic of 2 or more factors
- *Salmonellae* are grouped based on O antigen
- They are designated by Arabic numerals

Vi-Surface Ag

- Many strains of *S. typhi* have this antigen
- As it was related with virulence, labelled as Vi
- It is heat labile surface antigen, like K antigen of coliforms. It covers O antigen and renders the strain inagglutinable with O antisera, so bacilli inagglutinable with the O antiserum become agglutinable after boiling or heating for 1 hour

- Vi antigen tends to lose on serial subcultures
- It may act by coating bacterial surface and preventing the antibacterial opsonic effect of antibody
- In humans, strains with Vi antigen were found to cause clinical disease more consistently than those lacking the antigen
- It is poorly immunogenic so low titres of antibodies are produced following infection:
 - Detection of Vi antibodies is not helpful in diagnosis of cases, hence it is not used in Widal test
 - Total absence of Vi antibodies in a proven case of typhoid fever indicates poor prognosis
 - Antibodies usually disappear in convalescence and its persistence indicates development of the carrier state
 - It is used for epidemiological typing of *S. typhi* based on specific bacteriophages

■ Describe the different types of antigenic variations occurring in *Salmonella*.

Antigenic variations in *Salmonella* are:

1. H–O variation
2. Phase variation
3. V–W variation
4. S–R variation
5. Variation in O Ag

H–O Variation

- This variation is associated with the loss of flagella
- When salmonellae are grown on agar containing phenol (1:800), flagella are lost. This effect is phenotypic and the change is temporary; flagella reappear when they are subcultured on media without phenol
- A stable nonmotile mutant of *S. typhi* is 901—O strain, which is widely employed for preparation of O—agglutinable bacterial suspension
- Loss of flagella is not total; there occurs diminution in the number of flagella and quantity of H Ag
- When flagellated cells are few in number, to obtain population of motile cell, rich in H antigen, Craigie's tube is used

Craigie's Tube Technique

It consists of wide tube containing soft agar—0.2%. In the centre of the tube one more short, narrow tube open at both ends is embedded, which projects above the agar. Strain is inoculated into inner tube and after incubation, subcultures are taken from top of agar of outside tube.

Instead of Craigie's tube, 'U' tube may also be used in which sample is inoculated from one end and subculture is taken from the other end.

Phase Variation

- Flagellar antigen occurs in one of the two phases—phase I and phase II
- For serotyping of *Salmonella* isolates, it is necessary to identify H antigen of both phases
- Culture usually contains both phase antigens, but one of them predominates, so it is agglutinable with one of the phase antisera
- Culture in phase I can be converted to phase II by passing it through Craigie's tube containing phase I antiserum. Reverse conversion can be achieved by using phase II antiserum

V–W Variation

- Fresh isolates of *S. typhi* carry Vi antigen that masks O antigen
- These strains are agglutinable with Vi antiserum but not with O antisera. This is called 'V form'

- After a number of subcultures, the Vi antigen is completely lost; such cultures become inagglutinable with Vi antiserum and agglutinable with O antiserum. This is called 'W' form
- Intermediate stages, formed during loss of Vi antigen strain and that are agglutinable with Vi as well as O antisera are called 'VW' forms

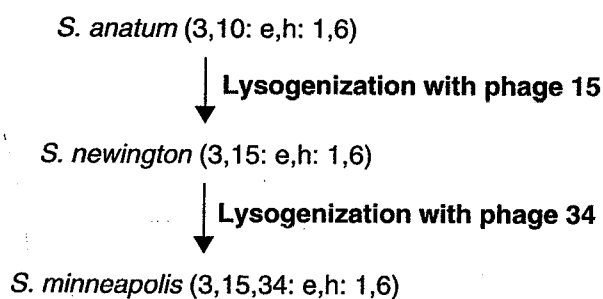
S-R Variation

- This variation is associated with (a) change in colony morphology from smooth to rough and (b) loss of virulence
- Colony becomes large, rough and irregular
- Strains are auto-agglutinable
- R forms are formed in laboratory strains that are maintained by subcultures or R form may occur by mutation

Variation in O Ag

Changes in structural formulae of O antigen may be induced by its lysogenization with some converting phages (Flowchart 38.1).

S. anatum is converted into *S. newington*, by lysogenization with phage 15 and *S. newington* gets converted to *S. minneapolis* by lysogenization with phage 34.



Flowchart 38.1 Changes in structural formulae of O antigen.

■ On what basis are salmonellae classified. Cite examples of some strains classified according to Kauffmann-White scheme.

1. Salmonellae have been classified into four subgenera based on biochemical reactions (lactose, dulcitol, malonate, d-Tartrate, salicin and KCN). Subgroup I is important cause of human and animal infections
2. Kauffmann-White scheme—it is a serological classification
 - Based on O Ag factors, salmonellae are classified into groups (Table 38.1) so, any strain possessing factor 2 is classified as group A and so on
 - Within each group, further differentiation is done based on phase I and phase II Ags

Table 38.1 Some important strains of salmonellae, serologically classified according to Kauffmann-White scheme

Serogroup	Serotype	O Ag	H phase I Ag	H phase II Ag
2-A	<i>S. paratyphi A</i>	1,2,12	a	—
4-B	<i>S. paratyphi B</i>	1,4,5,12	b	12
7-C1	<i>S. paratyphi C</i>	6,7(VI)	c	15
9-D	<i>S. typhi</i>	9,12(VI)	d	—

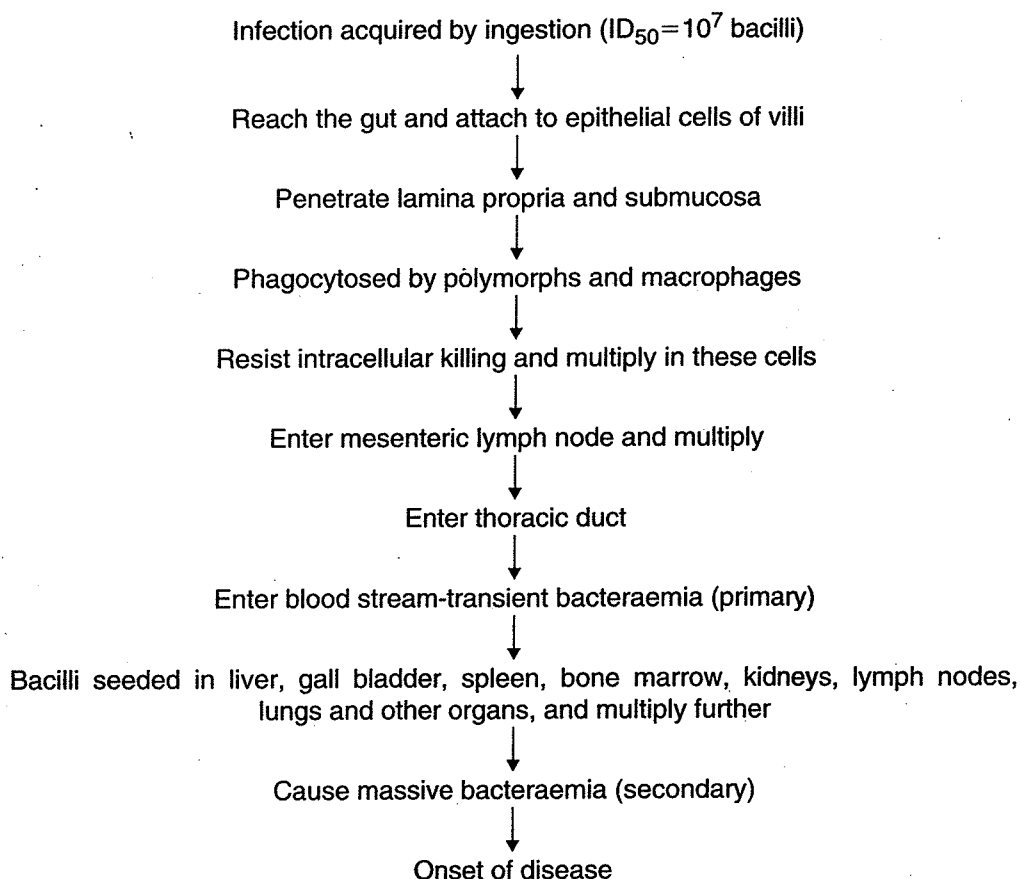
■ **Mention the diseases caused by salmonellae.**

Salmonellae cause the following clinical infections in humans:

- Enteric fever
- Septicaemia with or without local suppurative lesions
- Food poisoning or gastroenteritis

■ **Which microorganisms are responsible for enteric fever? Diagrammatically represent the course of disease development. Mention its clinical features and complications.**

- Enteric fever includes typhoid fever caused by *S. typhi* and paratyphoid fever caused by *S. paratyphi* A, B and C
- Course of disease development is presented in Flowchart 38.2
 - Bacilli multiply abundantly in gallbladder, as bile is good culture medium and discharged continuously in intestine where they involve Peyer's patches and lymphoid follicles of the ileum. These become inflamed, undergo necrosis and slough off, leaving typical typhoid ulcers. Ulceration may lead to perforation and haemorrhage. Intestinal lesions undergo healing in 3–4 weeks. This normally constitutes the course of the disease.



Flowchart 38.2 Course of development of enteric fever.

Clinical Features

Continuous type of fever (step ladder pyrexia), splenomegaly, rose spot rash, diarrhoea, anorexia, coated tongue, malaise and abdominal discomfort.

Complications

- Cholecystitis, bronchitis, pneumonia, meningitis, UTI-nephritis
- Chronic cholecystitis with gall stone

■ **Give the important features of septicaemia and gastroenteritis caused by *Salmonellae*.**

Salmonella Septicaemia

- The commonest cause of septicaemia is *S. choleraesuis*
- It is associated with suppurative lesions such as deep abscess, endocarditis, meningitis and pneumonia
- *Salmonella* can be isolated from blood

Salmonella Gastroenteritis

- It is usually a zoonotic disease
- It can be caused by any *Salmonella*, except *S. typhi*
- The commonest species involved is *S. typhimurium*, others are *S. enteritidis*, *S. haldar*, *S. anatum*, *S. newport*, and other species
- Infection occurs by ingestion of contaminated food
- Most frequent source is poultry, meat, milk and milk products, i.e. food of animal origin
- Food contaminated with droppings of lizard, rat and other small animals
- Salad or uncooked vegetable contaminated through manure or by handling
- Clinical features: Incubation period—24 hours—diarrhoea, vomiting, abdominal pain and fever

■ **Discuss the epidemiology of enteric fever.**

- Enteric fever is endemic in India
- *S. typhi* is the commonest cause of enteric fever followed by *S. paratyphi* A, B and C sequentially
- **Age:** Affects all ages
- **Source of infection:** Patient or carrier
- **Carriers:**
 1. **Convalescent carriers**—shed bacilli in faeces for 3 weeks to 3 months after clinical cure
 2. **Temporary and chronic carriers**—temporary carriers—shed bacilli for less than 1 year and chronic carriers—shed bacilli for more than 1 year
 3. **Faecal and urinary carriers**
 - i. Faecal—shed bacilli in faeces
 - ii. Urinary—shed bacilli in urine
 - iii. Food handlers are particularly dangerous, best example is Mary Mallon (Typhoid Mary), a cook who over a period of 15 years caused at least seven outbreaks affecting over 200 persons
 - iv. Milk-borne epidemics are rare due to pasteurization of milk
 - v. Water-borne epidemics and food-borne epidemics are known

■ **Describe the steps involved in laboratory diagnosis of enteric fever.**

Laboratory diagnosis of enteric fever includes:

1. Isolation and identification of bacteria from clinical specimens
2. Demonstration of antibodies
3. Demonstration of antigen

Isolation and Identification of Bacteria

Specimens

Blood, stool, urine, bone marrow, bile, pus from suppurative lesions and cerebrospinal fluid.

Collection

- **Blood:**

- Bacteraemia occurs early in disease and so blood cultures are positive in 90% cases in first week of fever, 75% in second week, 60% in third week and 25% after that
- It is collected by venepuncture with all aseptic precautions and added to transport media
- 5–10 ml blood should be added to 50–100 ml of bile broth (10-times dilution). This dilution helps in neutralizing bactericidal action of blood

- **Clot culture**

- 5–10 ml of collected blood is allowed to clot; serum is separated out and used for Widal test. The clot is broken up with sterile glass rod and added to blood culture medium containing streptokinase (it causes rapid lysis of clot releasing bacteria free)
- There are two advantages of this technique: 1. Rate of isolation of bacteria is more from clot culture and 2. Serum also becomes available for Widal test

- **Stool**

- Salmonellae are shed in faeces throughout the course and even in convalescence with varying frequency, so stool culture is also as valuable as blood culture
- Repeated sampling increases rate of isolation
- Portion of freshly passed faecal sample is added to enrichment media (so as to avoid overgrowth of the commensal) or to a sterile container and inoculated as early as possible on plating media

- **Urine**

- Salmonellae are shed infrequently in urine. So urine culture is less useful as compared to blood and stool cultures
- Repeated sampling improves rate of isolation
- Clean voided midstream urine sample is collected in a sterile container
- Sample is first centrifuged and then added to enrichment media

- **Bone marrow**

- It is positive in most cases even when blood culture is negative
- Bone marrow is collected with all aseptic precautions by bone marrow aspiration needle and added to transport media of blood culture

- **Bile:** Bile is obtained by duodenal aspiration. It may also be employed for detection of carriers. It should be collected in sterile container

- **CSF:** It is collected by lumbar puncture, with all aseptic precautions, in a sterile container

- **Sputum and pus:** These samples are collected in sterile containers and transported to laboratory

Transport

- Transport media are used for blood and faeces
- For blood and bone marrow—bile broth is used
- For faeces—buffered glycerol saline is used
- Other specimens should be transported to the laboratory as early as possible

Microscopy

It is important in samples like pus, cerebrospinal fluid and urine. Smear is prepared (after centrifugation in urine and CSF) and stained with **Gram's stain** to demonstrate Gram-negative bacilli

Culture

- **Blood and bone marrow:** After overnight incubation of bile broth, it is subcultured on blood agar and MacConkey's agar and selective media. Biphasic culture media can also be used for culture
- **Stool:** Part of stool sample is added to enrichment media such as selenite F or tetrathionate F broth to increase rate of isolation. Part of sample is inoculated on blood agar, MacConkey's agar and Wilson and Blair (it should be heavily inoculated)
- **Urine:** Sediment is inoculated into enrichment media and plating media
- **Bile, Sputum, Pus or CSF:** Inoculated directly on plating media

Colony characters

After overnight incubation at 37°C,

- Blood agar shows large 2–3 mm in diameter, circular convex, grayish-white, translucent colonies
- MacConkey's agar shows colourless, nonlactose fermenting colonies, which are processed further for identification
- Colony on Wilson and Blair medium helps in identification of species based on its colour
- If growth is not seen on first subculture of blood or bone marrow culture bottle then subculturing is repeated every day for 10 days and then only declared negative

Biochemical Reactions

- All species ferment glucose and mannitol with acid and gas except *S. typhi* (anaerogenic). *S. typhi* forms acid only
- All are citrate- and MR-positive (*S. typhi* and *S. paratyphi* A are citrate-negative)
- Urease—Negative
- H₂S—Positive except in *S. paratyphi* A and *S. cholerae-suis*

Serology

- A loopful of growth from nutrient agar is added to few drops of normal saline on slide to prepare emulsion
- An emulsion acts as control to show that strain is not autoagglutinable
- If *S. typhi* is suspected, a loopful of typhoid antisera factor-9 is added and observed for agglutination after rotating the slide manually
- Prompt agglutination indicates that isolate belongs to *Salmonella* group D. Then its identification as *S. typhi* is established by agglutination with flagellar antisera
- Fresh isolates of *S. typhi* are in V form and hence such strains do not agglutinate with O antisera
- Such strains are either tested with Vi antisera or growth emulsified in normal saline is boiled for 20 minutes and tested for agglutination with O antisera
- When isolate is nontyphoid *Salmonella*, it is tested for agglutination with O and H antisera for other groups

For identification of uncommon serotypes, the culture can be sent to reference centres. In India, the reference centre is at the Central Research Institute, Kasauli and for *Salmonella* of animal origin it is at the Indian Veterinary Research Institute, Izatnagar.

Antibiotic Sensitivity Testing

- It is done by Kirby–Bauer disc diffusion method
- Isolate is tested using chloramphenicol, ampicillin, ciprofloxacin, ofloxacin, sparfloxacin, ceftriaxone, cefotaxime, cefoperazone
- Multi-drug resistant *S. typhi* have been reported

Demonstration of Antibodies

Antibodies in patient's sera can be demonstrated by various tests such as:

- Widal test
- Indirect haemagglutination test
- Counter immunoelectrophoresis
- Enzyme-linked immunosorbent assay (ELISA)
- Radioimmunoassay (RIA)

Widal Test

Most widely used test, hence it is described in detail.

Principle

It is an agglutination test, which detects antibodies produced against *S. typhi*, *S. paratyphi* A and B.

Antigens used

- Four antigens are used in Widal test, these are:
 - H and O of *S. typhi*
 - H of *S. paratyphi* A
 - H of *S. paratyphi* B
- Paratyphoid O antigens are not used in the test as they cross-react with typhoid O antigens by sharing factor 12
- Antigens required can be prepared in laboratory or procured commercially

Procedure

- Widal rack with four rows of test tubes (for 4 Ags) is used for the test
- Equal volume of serial dilution of serum is added to all the test tubes
- Antigens are then added to respective rows
- Rack is incubated in water bath set at 37°C overnight
- Control tubes containing normal saline and antigens are also used to check auto-agglutination

Interpretation

- H agglutination—seen as loose cotton woolly clumps
- O agglutination—seen as granular disc-like pattern at bottom of test tube
- The following points should be considered while interpreting results of Widal test:
 - In typhoid endemic areas in developing countries active typhoid is suggested if the titre of H or O or both agglutinins are raised significantly
- Significant titres depend on the titre found in local healthy people
- Usually titres of 1:200 for H and 1:100 for O are considered significant
 - Single positive test result is not diagnostic of enteric fever and negative test result cannot rule out presence of enteric fever
 - Demonstration of rising titre in the tests made in the first and third week is highly significant than single test. But if the first sample is collected late in the disease, rise cannot be demonstrated. Antibody appears by 7th to 10th day of enteric fever so that negative test results may be obtained from samples collected early. Titre goes on increasing steadily till the third or the fourth week of enteric fever
 - Serum from individuals immunized with TAB vaccine show high titre antibodies against all antigens, while in a patient of enteric fever rise is only against one serotype
 - H agglutinins tend to persist for many months after vaccination but O agglutinins tend to disappear sooner. So rise in O indicates recent infection

- To demonstrate the serotype of infecting organism, H agglutinin is more reliable than O because serotypes have shared O Ag
- Persons with past history of enteric fever or immunization may develop anamnestic response during unrelated fever. This may be differentiated by repetition of the test after a week. Anamnestic response shows transient rise while in enteric fever the rise is sustained
- Patient treated with chloramphenicol shows poor agglutinin response
- False positive results may be obtained in nonspecific conditions because of fimbrial Ag, e.g.
 - Infection with other *Salmonella*
 - Immunological disorders, e.g. chronic liver disease
 - Rheumatoid arthritis
 - Multiple myeloma
 - Schistosomal infections

Demonstration of Antigens

- In early phase of disease, antigens are present in serum and urine of the patient
- Tests used for detection of antigens are:
 - Coagglutination
 - ELISA

■ Enumerate the ways in which carriers of *Salmonella* are detected.

Detection of carriers can be done in the following ways:

- Faecal carriers can be detected by isolation of bacilli from bile or faeces. Repeated sampling and chologogue purgative increase chances of isolation
- Urinary carrier can be detected by repeated urine culture
- Detection of Vi agglutinin indicates recent carrier. (It is a screening test, confirmation should be done by culture.)
- Sewer swab—it is important for tracing carriers in cities. Gauze pad left in sewers and drains are cultured and by tracing positive swab one may reach the house harbouring a carrier
- Another method of isolating *Salmonella* from sewage is filtration through millipore membrane and culturing membrane on highly selective media

■ Comment on the bacteriophage typing.

- Intraspecies classification of *S. typhi* for epidemiological purposes was made possible by bacteriophage typing
- This phage typing depends on Vi Ag, strains without Vi Ag are untypable
- National phage typing centre is located at Lady Hardinge Medical College, New Delhi
- Phage type A and E are commonly present throughout India

■ Describe the prophylactic measures that should be taken to check *Salmonella* infection.

Prophylaxis can be done in the following ways:

General Measures

Methods for control of enteric fever are:

- Proper sewage disposal
- Safe water supply
- Handling food hygienically
- Periodic examination of food handlers to rule out carrier state

Vaccines

1. Parenteral Vaccines

- TAB vaccine: It is a heat-killed, phenol-preserved whole cell vaccine with mixture of:
 - *S. typhi*: $1000 \times 10^6/\text{ml}$
 - *S. paratyphi* A: $750 \times 10^6/\text{ml}$
 - *S. paratyphi* B: $750 \times 10^6/\text{ml}$
- Schedule: 2 doses of 0.5 ml each at an interval of 4–6 weeks followed by booster every 3 years
- Efficacy: 70–90%

2. TA Vaccine

- Because of prevalence of only *S. typhi* and *S. paratyphi* A in some area, and absence of *S. paratyphi* B, TA vaccine is used instead of TAB, e.g. in India, TAB is replaced by divalent typhoid–paratyphoid A vaccine
- Schedule: 2 doses intramuscularly 4–6 weeks apart

3. Vaccine of Purified Vi Ag (Typhim Vi)

- It contains purified Vi Ag from *S. typhi* Ty 2
- It is given by intramuscular route
- Single dose of 25 μg
- Efficacy: 64% to 72%
- Protection starts after 2–3 weeks and lasts for at least 3 years

4. Oral Vaccines

- Typhoral: It is an oral vaccine containing stable mutant of *S. typhi* strain—Ty 21a lacking enzyme UDP–galactose 4-epimerase
- It is an enteric coated capsule containing viable lyophilised mutant bacilli
- On ingestion, it initiates infection but self-destructs after four or five cell division, hence cannot induce illness
- Schedule: 3 doses of one capsule orally, an hour before food with water or milk on days 1, 3, 5
- Protection starts after 2–3 weeks and lasts for 3 years, after which booster may be needed

■ How should *Salmonella* infections be treated?

- Treatment should be guided by antibiotic sensitivity testing because of emergence of multiple drug resistance
- Chloramphenicol, ampicillin, co-trimoxazole, amoxycillin, ciprofloxacin and other fluoroquinolones can be used

39

Chapter

Enterobacteriaceae III: *Shigella*

■ **Mention the disease caused by *Shigella*. How did it get its present name? Similarly mention other species of this genus.**

- The genus *Shigella* is a cause of bacillary dysentery in human beings, characterized by the passage of loose motions mixed with blood and mucus
- The genus has been labelled after Shiga, the person who isolated the first member of genus in 1896—*Shigella shiga*, i.e. Type I

Other species of *Shigella* are:

- *Sh. flexneri*—described by Flexner
- *Sh. sonnei*—described by Sonne
- *Sh. boydii*—described by Boyd

■ **State the characteristic morphological features of *Shigella*.**

Morphological Features

- Gram-negative bacilli
- Size: $2-4 \mu \times 0.6 \mu$
- Nonmotile, nonsporing and noncapsulated
- Some strains possess fimbriae of Type I

■ **Mention the distinguishing cultural characteristics of *Shigella*; include media and colony characters.**

Cultural Characters

- Aerobe and facultative anaerobe
- Optimum pH 7.4
- Optimum temperature 37°C
- Can grow on ordinary media

Media and Colony Characters

- **Nutrient agar or blood agar:** Large, 2–3 mm in diameter, circular convex, smooth, translucent, gray colonies
- **MacConkey's agar:** Nonlactose fermenting (colourless), except *Sh. sonnei*, which is a late lactose fermenter, becomes pink when incubation is prolonged beyond 24 hours
- **Selective media:**
 - **Deoxycholate citrate agar**—colonies are colourless, except *Sh. sonnei*, which is a late lactose fermenter and therefore produces pink colonies after 24 hours
 - **Xylose lysine deoxycholate medium (XLD)**—colonies are red in colour
 - **Salmonella Shigella agar** (medium containing high concentration of bile salt, which inhibits Gram-positive bacteria and coliforms, also contains lactose, neutral red and indicator for H_2S)—colourless colonies are formed

- **Enrichment media:** These media maintain normal flora in a prolonged lag phase, e.g. **selenite F broth** (sodium selenite inhibits other coliforms while permit shigellae and salmonellae to grow) and **Gram-negative broth** (inhibits Gram-positive bacteria, but less inhibitory to Gram-negative bacteria as compared to selenite F broth)

■ Which biochemical reactions characterize *Shigella* spp.?

Important biochemical reactions are:

- Catalase—positive, except *Sh. dysenteriae* Type I
 - Oxidase—negative
- | | | | | | | |
|---|----|----|---|--------|------------------|-----|
| I | MR | VP | C | Urease | H ₂ S | PPA |
| V | + | - | - | - | - | - |
- (+ = positive, - = negative)
- Produce acid from glucose
 - Mannitol—fermented by all, except *Sh. dysenteriae*
 - Lactose—not fermented, except *Sh. sonnei*, which ferments lactose late (LLF)

■ Mention the ways in which *Shigella* spp. can be killed?

- *Shigella* spp. are killed by heat at 56°C in 2 hours and by treating with 1% phenol for 30 minutes
- They are killed by chlorination
- They are killed in stools that are allowed to become acidic through growth of coliforms and they die within few hours but survive for several days in faeces kept in nonacidic conditions such as in buffered glycerol saline
- *Sh. sonnei* is more resistant to adverse environmental conditions as compared to other species

■ Classify shigellae.

Based on biochemical reactions and antigenic structure, shigellae are classified into four groups. These are:

- Group A: *Sh. dysenteriae*—Mannitol nonfermenting
 - Group B: *Sh. flexneri*
 - Group C: *Sh. boydii*
 - Group D: *Sh. sonnei*
- } Mannitol fermenting

■ Describe the antigenic structure of shigellae.

Antigenic structure of shigellae:

- **Group A: *Sh. dysenteriae***—It is divided into 15 serotypes
 - Type 1—known as *Shigella shiga*
 - Type 2—known as *Sh. schmitzi*
 - Type 3–7—are called Large and Sach's group described in India
- **Group B: *Sh. flexneri***—Based on type specific (I–VI) and group specific (1 to 8) antigens, it is divided into six serotypes (1–6) and several subtypes 1a, 1b, 2a, 2b, 3a, 3b, 4a, 4b, 5a, 5b
 - In addition to six serotypes, there are two antigenic variants X and Y
 - Three biotypes of serotype 6 are:
 - Boyd 88, which ferments glucose and mannitol with acid
 - Manchester, which ferments glucose and mannitol with acid and gas
 - Newcastle, which ferments glucose with acid and with or without producing gas but not the mannitol

- **Group C:** *Sh. boydii*—Biochemically similar to *Sh. flexneri* but antigenically different 19 serotypes
- **Group D:** *Sh. sonnei*—Ferments lactose and sucrose late
 - only one serotype but occurs in two phases- I and II
 - classified into 26 colicin types for epidemiological purpose

■ Describe the toxins produced by *Shigellae*.

Toxins produced by shigellae and their features are, as follows:

1. Endotoxin

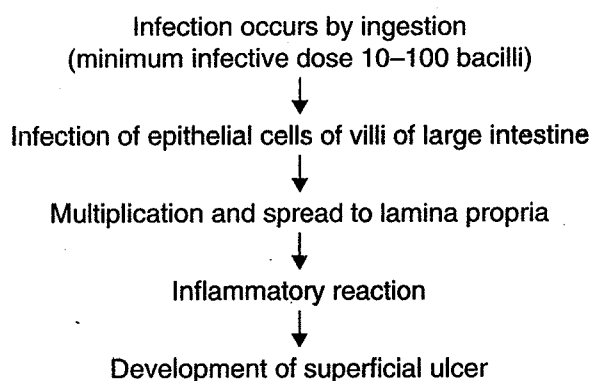
- All shigellae release endotoxin after autolysis
- It is thermostable lipopolysaccharide (LPS) of cell wall
- It acts on intestinal wall causing diarrhoea and ulcers

2. Exotoxin

- It is produced only by *Sh. dysenteriae* Type I
- It acts as enterotoxin and neurotoxin
- Enterotoxin acts on intestinal mucosa causing transudation of fluid in lumen
- Neurotoxin damages endothelial cells of small blood vessels of central nervous system, which result in neurological complications like polyneuritis, coma, meningism
- Some strains of *Sh. dysenteriae* also produce cytotoxin, which acts on Vero cells and is known as verocytotoxin

■ Write the path of disease development triggered by ingestion of *Shigella* spp.

- Path of disease development, triggered by ingestion of *Shigella* spp., is presented in Flowchart 39.1.



Flowchart 39.1. Disease development in infection of *Shigella* spp.

■ Describe in brief the disease caused by *Shigella*.

- Shigellae cause **shigellosis**
- *Sh. flexneri* is the commonest species followed by *Sh. dysenteriae* and *Sh. sonnei*
- *Sh. boydii* has been isolated least frequently
- **Symptoms:** The symptoms vary from acute dysentery characterized by presence of blood and mucus in stools to mild diarrhoea with watery stools
- **Sources:** Human beings—infected cases (rarely carriers)
- **Modes of transmission:**
 - **Fingers**—through contaminated fingers
 - **Fomites**—through door handles, water taps, lavatory seats
 - **Flies**—mechanical vectors—can transmit organisms from faeces to food
 - **Food and water**—through contaminated food and water

■ **Give the clinical features and complications of *Shigella* infection.**

Clinical Features

- Incubation period: 2–3 days
- Typical presentation is
 - Passage of bloody mucoid stools
 - Sudden onset, griping pain, tenesmus, pyrexia, prostration
 - Sometimes convulsions
 - Severity varies with species. Severe form of dysentery is caused by *Sh. dysenteriae*; however, *Sh. sonnei* causes mild diarrhoea with watery stools

Complications

These often seen in *Sh. dysenteriae* Type - I. The important complications are:

1. Arthritis
2. Toxic neuritis
3. Conjunctivitis
4. Parotitis
5. Intussusception in children
6. Haemolytic uraemic syndrome in severe cases

■ **Write in brief the laboratory diagnosis of *Shigella* infection.**

Specimens

Fresh stool samples

Swab from ulcer

Collection

- Stool is collected in bedpan and portion of it containing mucus flakes is transferred to transport media
- Swab from ulcer is collected by sigmoidoscopic examination

Transport

Specimen is transported in transport medium such as buffered glycerol saline, pH 7.0–7.4

Microscopic Examination

- **Wet preparation**—stool shows presence of plenty of pus cells, RBCs and nonmotile bacilli

Culture

- Stool is cultured on blood agar, MacConkey's agar, DCA plates
- Incubation: Overnight at 37°C
- **Colonies on blood agar:** Large, circular, gray, convex, opaque, translucent
- **MacConkey's agar and DCA plates:** Nonlactose-fermenting (NLF) colonies (colourless colonies)
- **SS agar:** Colonies are colourless without blackening
- Stool samples may also be added to enrichment media and subcultures are made on plating medium after 8–12 hours and incubated overnight at 37°C and colony morphology is studied. Typical colonies are processed further for identification by routine procedures as follows:
 - Gram-stained smear of colony shows Gram-negative bacilli
 - Motility—nonmotile bacilli
 - Biochemical reactions

Biochemical Reactions

- Sugar fermentation reactions (glucose, lactose, sucrose and mannitol fermentation)—Mannitol is fermented by all except *Sh. dysenteriae*
 - Lactose and sucrose are fermented late by *Sh. Sonnei* (takes more time to ferment)
 - Glucose is fermented by all species with acid only
 - Catalase—Positive
 - Oxidase—Negative
- | | | | | | |
|---|----|----|---|--------|------------------|
| I | Mr | VP | C | Urease | H ₂ S |
| V | + | - | - | - | - |
- (+ = positive, - = negative)

Serology

Confirmation by slide agglutination test with polyvalent and monovalent antisera.

Antibiotic Susceptibility Testing

It is performed by Kirby-Bauer disc diffusion method, using ampicillin, nalidixic acid, tetracycline, ciprofloxacin, and other fluoroquinolones.

■ What treatment measures are recommended for *Shigella* infection?

Shigella infection can be treated in the following ways:

- Replacement of fluids by oral or IV route as per requirement
- Antibiotic treatment—ampicillin, tetracycline, nalidixic acid, norfloxacin and ciprofloxacin
- As multidrug resistant shigellae have been identified, the treatment should be guided by antibiotic susceptibility testing

■ Mention three most important ways of preventing and controlling *Shigella* infection.

Prevention and control can be achieved by:

- Improvement of environmental sanitation
- Improvement of personal hygiene
- Protection of food from flies

40

Chapter

Pseudomonas and *Burkholderia*

■ Mention the species of *Pseudomonas*.

Species of *Pseudomonas* are:

- *P. aeruginosa*—also called *Pseudomonas pyocyanea*, *Bacillus pyocyaneus*
- *P. putida*
- *P. fluorescence*
- *P. stutzeri*

■ State the salient morphological features of *Pseudomonas aeruginosa*.

Morphological Features

- Slender Gram-negative bacillus
- Size: $1.5-3 \mu \times 0.5 \mu$
- Arranged singly or in pairs or short chains
- Actively motile by one or two polar flagella
- Nonsporing and noncapsulated but many strains show presence of mucoid slime layer

■ Mention the cultural characteristics of *P. aeruginosa*; include media and colony characters.

Cultural Characters

Can grow on ordinary media

- Obligate aerobe
- Optimum pH 7.2–7.4 and optimum temperature 37°C

Media and Colony Characters

- **Nutrient agar:** Colonies are large, 2–3 mm in diameter, irregular, oval around long axis, convex, smooth, opaque and emit characteristic fruity odour or earthy smell, possess metallic shin along with bluish-green diffusible pigment (Fig. 15.1)
- Mucoid colonies are especially observed when isolate is from patients of cystic fibrosis
- **Blood agar:** Haemolytic colonies
- **MacConkey's agar:** Nonlactose-fermenting colonies (NLF)

■ Highlight the features of pigments produced by *P. aeruginosa*.

P. aeruginosa produces a number of pigments. These are:

- **Pyocyanin**
 - It is a bluish-green, diffusible phenazine pigment soluble in water and chloroform
 - It is an identifying feature of *P. aeruginosa* as it is not produced by any other species
 - It inhibits growth of many other bacteria and make *P. aeruginosa* as a dominant bacterium in mixed infections.

- **Fluorescein**
 - Also called pyoverdin. It is a greenish-yellow pigment, soluble in water but not in chloroform
 - It gives yellowish tinge to culture, which can be better observed when plate is examined under UV light
 - In old cultures it may be oxidized to yellowish-brown pigment
- **Pyorubin**
 - It is a bright red phenazine pigment soluble in water and insoluble in chloroform
- **Pyomelanin**
 - It is a brown black pigment

■ **Mention the biochemical reactions of *P. aeruginosa*.**

Biochemical Reactions

- It is a nonfermenter, metabolism is oxidative
- It has to be tested on oxidation–fermentation media, as acid produced by oxidative pathway is less than that produced by fermentation. It utilizes only glucose oxidatively

I	M	Vi	C	H ₂ S
–	–	–	+	–

(– = negative, + = positive)

- All strains give prompt oxidase-positive reaction within 30 seconds
- Reduce nitrate to nitrite
- Catalase—positive
- Gelatinase—negative

■ **Write a note on 'resistance' of *P. aeruginosa*.**

- *Pseudomonas aeruginosa* survives well in wet environment and is susceptible to heat
- It is resistant to common disinfectants and antiseptics, and can grow in some disinfectant solutions such as iodine, hexachlorophane, soap solutions, quaternary ammonium compounds, therefore cetrime can be incorporated in selective media
- It has considerable natural resistance to many antibiotics

■ **Describe the antigenic structure of *P. aeruginosa*.**

Pseudomonas aeruginosa has two antigens. These are:

- **O antigen**
 - 19 group-specific O antigens
 - On this basis it can be divided into 27 serotypes
 - Serotypes 6 and 11 predominate in clinical specimens and type 11 is mainly responsible for most hospital-acquired infections
- **H antigen**
 - It has two heat labile H Ag

■ **Mention the virulence factors of *P. aeruginosa*.**

Virulence factors of *P. aeruginosa*, toxins and enzymes are as follows:

- Extracellular products
 - **Pyocyanin** inhibits mitochondrial enzymes in tissue and causes disruption and cessation of ciliary beat on ciliated nasal epithelium, which favours colonization by organisms by avoiding its clearance from respiratory mucosa by host defenses

- Extracellular enzymes and haemolysins
 - It produces proteases (general protease, alkaline protease and elastase), haemolysins, and lipases, which help in formation of local lesion
 - **Elastase** digests elastin of arterial wall leading to destructive vascular lesions along with haemorrhage
 - It also inactivates some complement components
 - It causes tissue necrosis along with alkaline protease
 - **General protease** is collagenase that hydrolyses collagen
 - **Proteases** aid in spread of organism in tissue
 - **Haemolysins** are mildly toxic, heat labile, phospholipase C and cause redness and induration on intradermal injection
 - **Lipases** degrade a variety of fats
- **Exotoxin**
 - It produces two exotoxins, A and S. A is polypeptide that inhibits protein synthesis
- **Endotoxin**
 - It is a lipopolysaccharide. It exhibits all the properties of enterobacterial lipopolysaccharides (LPS) including pyrogenic action and Shwartzman reaction

■ **Write a short note on bacteriocin produced by *P. aeruginosa* and method of bacteriocin typing.**

- Bacteriocin produced by *P. aeruginosa* is known as pyocin. It is an antibiotic-like substance
- There are three types of pyocins: R, F and S
 - R—resembles tail of contractile phages
 - F—are rod shaped, resemble tail of noncontractile phages
 - S—has no discernible structure
- Ability to produce pyocin is found in 90% strains
- Pyocin typing is the most common typing method of *P. aeruginosa*

Typing Method

- Strain inoculated as wide band across a plate, incubated overnight at 30°C for pyocin production
- Growth is then removed by scraping
- Residual growth killed by exposure to chloroform vapours
- 8 indicator strains—No. 1–8—are cross-streaked in parallel lines at right angle to original inoculum followed by incubation at 37°C
- Pattern of inhibition of indicator strains determines the type of strain

■ **Describe the pathogenicity of *P. aeruginosa*.**

- *P. aeruginosa* can cause infection in community; outside the hospital as well as hospital acquired infections (HAI)
- Its ability to persist in moist environment of wards, bedroom, kitchen, equipments-like respirator, scopes, articles-like bedpan, medicines such as eye/ear drops, lotions, ointment, distilled water and floor, favours its role in HAI, such as
 - Infections of wound, bedsores
 - Eye infections
 - Urinary tract infections following catheterization
 - Acute purulent meningitis following lumbar puncture or cranial injury
 - Necrotizing pneumonia following colonization of ventilators in debilitated persons
 - Septic arthritis and endocarditis in drug addicts

- Septicaemia and endocarditis in debilitated persons
- Infection of nail bed following excessive exposure to detergents and water
- Infantile diarrhoea and sepsis
- **Shanghai fever**—self-limited fever
- Can cause acute necrotizing vasculitis leading to haemorrhagic infarction of skin and internal organs

■ **Describe the laboratory diagnostic procedures and tests for determining infections by *P. aeruginosa*.**

Specimens

Pus, wound swab, urine, sputum, blood, etc., depending on the site involved.

Collection

Samples should be collected in sterile containers by using appropriate procedures.

Transport

Transported immediately to Microbiology laboratory.

Microscopic Examination

- **Gram stain**—shows pus cells along with Gram-negative bacilli—noncapsulated and nonsporing, arranged singly or in short chains

Culture

- Specimen is plated on blood agar and MacConkey's agar
- **Blood agar:** Large, irregular, smooth, opaque colonies, with fruity odour, bluish-green pigment and haemolysis
- **MacConkey's agar:** Nonlactose fermenting (colourless) colonies
- **Cetrimide agar:** It is required especially for isolation of *Pseudomonas* from stool or wound swab where mixed flora is present

Biochemical Reactions

- Attacks glucose oxidatively
- Oxidase—positive
- Citrate—positive

Typing

Serotyping and pyocin typing.

Antibiotic Susceptibility Testing

It is performed by Kirby–Bauer disc diffusion method using amikacin, carbenicillin, azlocillin, ticarcillin, cefotaxime, ceftazidime, gentamicin, tobramycin, ciprofloxacin, cefoperazone, carbapenem, etc.

■ **Mention the distinctive features of *Stenotrophomonas maltophila* (syn. *Pseudomonas maltophila*).**

Distinctive features of *S. maltophila* are as follows:

- It acidifies maltose—hence named maltophila. Also acidifies glucose, lactose and sucrose
- It is a saprophyte and can cause opportunistic infections such as wound, urinary tract infections and septicaemia

- It is oxidase-negative
- Infection responds to cotrimoxazole and chloramphenicol

■ **Name the species of *Burkholderia*.**

Species of *Burkholderia* are:

- *B. mallei*
- *B. pseudomallei*
- *B. cepacia*

■ **Write the salient features of *B. pseudomallei*, *B. mallei* and *B. cepacia*.**

B. pseudomallei

- It was identified by Whitmore as the etiological agent of **melioidosis**, a glanders-like disease of rodents that is transmissible to humans, therefore it is also called *B. whitmori*
- It is a small, Gram-negative bacillus, about $2.5 \mu \times 0.5 \mu$ and motile
- It causes human infection through inhalation, inoculation, rarely by ingestion or by bite of haematophagous insects (from animals)
- Clinical features: In humans clinical features are:
 - Benign pulmonary infection resembling tuberculosis
 - Multiple abscesses in internal organs
 - Fulminating septicaemia
- Laboratory diagnosis
 - It is done by microscopic demonstration and isolation of bacilli from exudates, urine, pus and blood
 - Ab detection—detection of IgM and IgG by ELISA and indirect haemagglutination test

B. mallei

- It is a small Gram-negative but nonmotile bacillus; obligate animal pathogen transmitted to man causing **glanders**
- Humans are infected via skin abrasion or wounds, which come in contact with discharges of sick animals
- Clinical features: Acute febrile illness, abscess in respiratory tract and skin
- Laboratory diagnosis: It is done by microscopic demonstration and isolation of bacilli

B. cepacia

- It is a slender, motile, Gram-negative bacillus
- On prolonged incubation colony becomes reddish purple
- It is increasingly isolated from cases of cystic fibrosis
- Clinical features: It can cause urinary, respiratory, wound infection, peritonitis, endocarditis and septicaemia
- Laboratory diagnosis: It is done by isolation of bacilli from specimens

41

Chapter

Vibrio

■ Name the important species of *Vibrio*.

The important species of *Vibrio* are:

- *V. cholerae* is the most important species, first isolated by Koch (1883).
- *V. parahaemolyticus*
- *V. vulnificus*

■ Mention the morphological characteristics of *Vibrio cholerae*.

Morphological Characteristics

- Gram-negative bacilli
- Short, curved like comma with rounded or pointed ends (Fig. 41.1)
- Size: $1.5-2.5 \mu \times 0.2-0.4 \mu$
- S-shaped and spiral forms are seen due to cells lying close to each other
- In stained films of mucus flakes, they appear in parallel rows giving "Fish in stream" appearance
- Actively motile with single polar flagellum—shows darting motility
- Nonsporing and noncapsulated

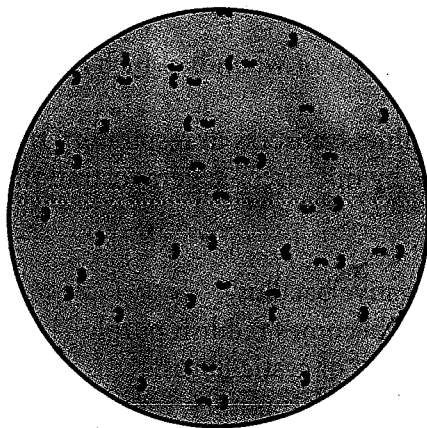


Fig. 41.1 *Vibrio*.

■ Write the types of media used and colony characteristics of *V. cholerae*?

Cultural Characters

- Aerobe and facultative anaerobe
- Grow on ordinary media
- Optimum temperature 37°C (range $16^{\circ}-40^{\circ}\text{C}$) and optimum pH 8.2 (range 8.2–9.5)

Media and Colony Characters

- **Nutrient agar:** Large, round colonies, 1–2 mm in diameter, translucent with bluish tinge in transmitted light

- **Blood agar:** Greenish zone of discolouration around colony, which later on becomes clear due to haemodigestion
- **MacConkey's agar:** Nonlactose fermenting colonies (colourless), become pink on prolonged incubation (late lactose fermenting)

Special media for *Vibrio cholerae* are:

Selective media or plating media

- **Thiosulphate, citrate, bile salt, sucrose (TCBS) agar** (Fig. 41.2) – Forms yellow colonies because of sucrose fermentation and indicator bromothymol blue and turn green on continued incubation
- **Alkaline bile salt agar (BSA)** – Colonies are similar to that on nutrient agar
- **Mansur's gelatin taurocholate trypticase tellurite agar (GTTTA)** – Small, translucent with gray-black centres and turbid halo

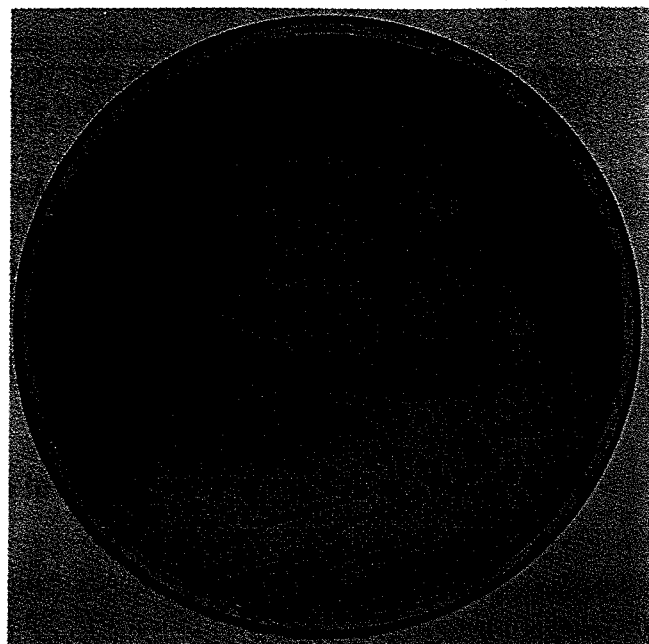


Fig. 41.2 Colonies of *Vibrio* on TCBS. (Source: Journal of Textbook of Diagnostic Microbiology, Figure 21-4, 2007.)

Transport media or holding media

- **Venkatraman–Ramakrishnan medium (V–R medium)** – It preserves vibrio for several weeks and prevents overgrowth of other organisms
- **Cary–Blair medium** – It is used for *Salmonella*, *Shigella* and *Vibrio*
- **Autoclaved seawater**

Enrichment media

- **Alkaline peptone water** – High pH suppresses growth of commensals and allows growth of vibrio – Can also be used as a transport medium
- **Taurocholate tellurite peptone water** – Incubation of 6–8 hours in enrichment media is sufficient

■ Describe the reactions that are used for identifying *V. cholerae*.

Biochemical Reactions

- Catalase and oxidase—positive
- L G M S I M VP C
– + + + + – V –
(+ = positive, – = negative, V = variable)
- Urease and H₂S—negative
- Nitrate reduction—positive
- Liquefy gelatin

Cholera Red Reaction

When 24-hour old liquid culture is mixed with few drops of concentrated sulphuric acid, red-pink colour develops due to formation of nitroso-indole. The test is positive in *V. cholerae*

String Test

When growth is mixed with 0.5% sodium deoxycholate in saline, suspension loses its turbidity and becomes mucoid to form string when loop withdrawn from suspension. Test is positive in *V. cholerae*

Haemolytic Reaction

- Equal volumes of broth culture and 1% sheep erythrocytes are mixed and incubated for 2 hours at 37°C then kept in refrigerator for overnight at 4°C and examined for haemolysis
- Classical *Vibrio*—nonhaemolytic
- El Tor *Vibrio*—haemolytic

■ Mention the factors governing survival of *V. cholerae*.

Vibrio cholerae are:

- Susceptible to heat and drying
- Susceptible to pH less than 5
- Resistant to alkalinity
- Survival in water is affected by many factors, such as presence of phages and pH
- El Tor *Vibrio* survives longer than classical
- Survive in clean tap water for 30 days, in night soil for several days and on fruits for 1–5 days

■ Which antigens are present in *V. cholerae*?

Antigens present in *V. cholerae* are:

1. **Somatic O Ag:** On the basis of O Ag, there are 139 serogroups.
2. **Flagellar H Ag**

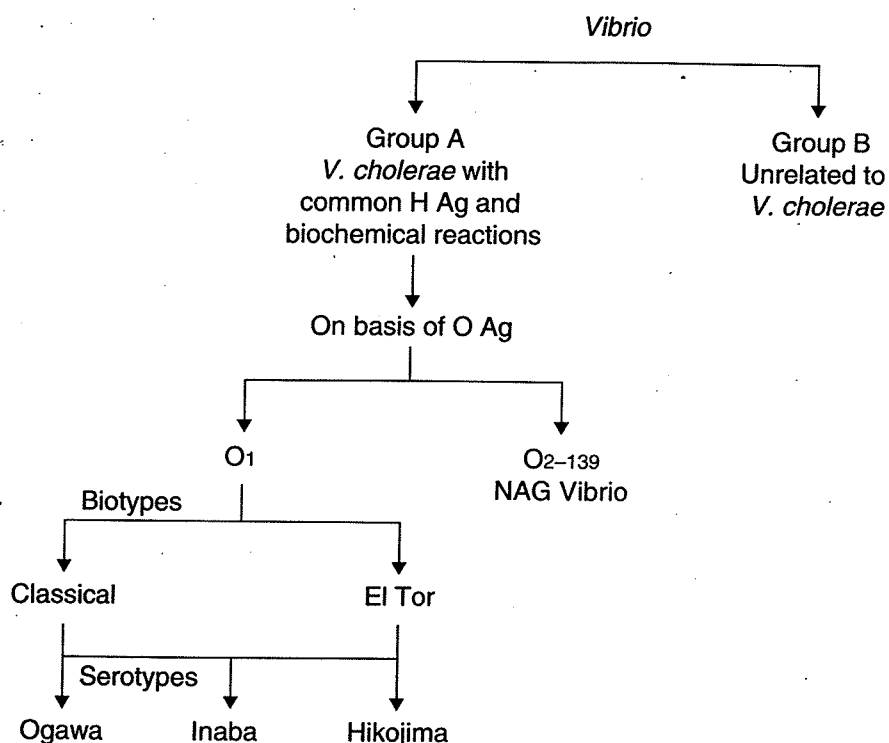
■ Comment on bacteriophage typing of *V. cholerae*.

- Classical strains are divided into I to V phage groups by using Mukherjee's four phages
- Later on one more phage I is employed and now 6 phage-groups have been identified

■ Classify *Vibrio* according to Gardner and Venkataraman's system of classification.

Classification of *Vibrio* according to Gardner and Venkataraman's system of classification is given in Flowchart 41.1.

- Group A includes *V. cholerae* with general biochemical similarity with *V. cholerae* and B are unrelated to *V. cholerae*
- Group A have common H Ag
- On the basis of O Ag
 - There are O₁ to O₁₃₉ serogroups (Flowchart 41.1)
 - *V. cholerae* O₂ to O₁₃₉ are called nonagglutinating *Vibrio*
 - *Vibrio* O₁ are further divided into 2 biotypes, Classical and El Tor, based differences enumerated in Table 41.1
 - Each biotype is further divided into Ogawa, Inaba and Hikojima on the basis of minor O Ags (ABC):
 - Ogawa AB
 - Inaba AC
 - Hikojima ABC



Flowchart 41.1 Gardner and Venkatraman's classification of *Vibrio*.

Table 41.1 Differences between biotypes of *V. cholerae*

	Classical	El Tor
1. Voges-Proskauer test	—	+
2. Agglutination of chick RBCs	—	+
3. Haemolysis of sheep RBCs	—	+
4. Sensitivity to polymyxin B	+	—
5. Phage V susceptibility	—	+
6. Phage IV susceptibility	+	—

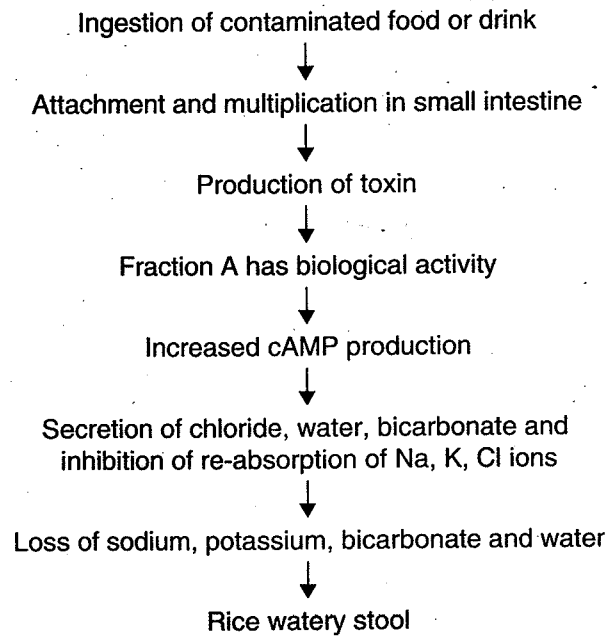
+ = positive, — = negative.

■ Diagrammatically depict the course of disease development in *V. cholerae* infection. Also write the clinical features of the disease.

Development of disease in *V. cholerae* infection is given in Flowchart 41.2.

Clinical Features

- Profuse painless watery diarrhoea and effortless vomiting leads to severe dehydration causing haemoconcentration, anuria and hypovolaemic shock
- **Complications**
 - Muscular cramps
 - Renal failure
 - Cardiac arrhythmia
 - Pulmonary oedema



Flowchart 41.2 Course of disease development in *V. cholerae* infection.

■ **Describe the laboratory procedures and examinations employed in diagnosing *V. cholerae* infection.**

Collection of Specimens

- **Stool:** It is an ideal specimen, collected by inserting sterile rubber catheter and allowing stool to flow into sterile container
- **Rectal swab:** Cotton swabs moistened with transport media are inserted in rectum, left at the place for few seconds to allow absorption of fluid. They are used for patients in convalescence or carriers

Transport

- If there is delay in plating, Venkatraman–Ramakrishnan or Cary–Blair transport medium is used
- If transport medium is not available blotting paper soaked in watery stool and packed in plastic envelope should be sent to laboratory
- If there is delay in culture, enrichment media are used as they save time required for isolation, e.g.
 1. Alkaline peptone water
 2. Taurocholate tellurite peptone water

Microscopic Examination

For rapid diagnosis, the methods are:

- Demonstration of motility by *hanging drop preparation* and its inhibition by antisera and distilled water
- *Direct immunofluorescence test*

Culture

- Direct plating and culture after enrichment increases the chances of isolation of bacteria
- In addition to routine media such as blood agar, MacConkey's agar, selective media should also be used
- **Nutrient agar:** Large, round colonies, 1–2 mm in diameter translucent, with bluish tinge in transmitted light

- **Blood agar:** Greenish zone of discolouration around colony, which later on becomes clear due to haemodigestion
- **MacConkey's agar:** Nonlactose fermenting (colourless), become pink on prolonged incubation (late lactose fermenting)
- **Thiosulphate, citrate, bile, sucrose agar (TCBS)**—yellow colonies
 - Typical yellow colonies are processed for identification by using biochemical tests

Biochemical Tests

- Catalase and oxidase—Positive
- L G M S I M Vi C
 – + + + + – V –
 (+ = positive, – = negative, V = variable)
- Urease, H₂S—Negative
- Other tests used for identification, which help to differentiate biotypes include the following (Table 41.1):
 - Cholera red reaction
 - String test
 - Haemolytic reaction
 - Susceptibility to O/129

Serology

- Bacterial suspension is tested by adding O subgroup I serum
- If positive, tested with Ogawa and Inaba sera to detect serotype
- If the colony is not agglutinated by O subgroup I serum, it is then tested with antisera to antigen, if it shows agglutination, reported as non-O₁ *Vibrio cholerae*
- Specific antisera to O₂₋₁₃₉ are available and can be used for its detection

Antibiotic Sensitivity Testing

It is performed by Kirby–Bauer disc diffusion method

■ How are carriers of *Vibrio* detected?

Detection of carriers is done by:

- Repeated stool culture
- Stool collected after purgative
- Bile culture
- Serological tests

■ What treatment measures are recommended in case of *V. cholerae* infection?

Treatment recommended includes:

- Fluid and electrolyte replacement—to correct severe dehydration by oral rehydration therapy either alone or supplemented by intravenous fluids
- Antibiotics—usually reduce *Vibrio* excretion—tetracycline is used

■ What are halophilic vibrios?

Vibrios that need high concentration of sodium chloride and cannot grow in absence of it are called halophilic vibrios, e.g.

1. *V. parahaemolyticus*
2. *V. alginolyticus*
3. *V. vulnificus*

Naturally, they exist in seawater and have a marine life.

■ **Mention the salient features of *V. parahaemolyticus*, *V. alginolyticus* and *V. vulnificus*.**

V. parahaemolyticus

- **Morphology:** Similar to *V. cholerae* except that they are capsulated and show bipolar staining
- **Culture:** They can grow only on media with 2–4% sodium chloride
 - They can tolerate 8% NaCl but not 10%
 - On TCBS they form green colonies due to sucrose nonfermentation
- **Pathogenicity:** All strains are not pathogenic, only those strains, which show “**Kanagawa phenomenon**”, can cause food poisoning
- **Kanagawa phenomenon:** When grown on high salt blood agar, they produce haemolysis
- Association with marine food, abdominal pain, diarrhoea, vomiting and fever characterizes food poisoning

V. alginolyticus

- **Morphology:** Morphologically resembles *V. parahaemolyticus* in many respects
- **Culture:** It is the most halophilic *Vibrio*, can tolerate 10% NaCl. It is sucrose fermenter
- **Pathogenicity:**
 - It is found in sea fish
 - It causes infection of eyes, ears, wound in humans acquired by swimming in seawater

V. vulnificus

- **Morphology:** Morphologically resembles *V. parahaemolyticus*
- **Culture:** It can tolerate 8% NaCl. It is a lactose fermenter
- **Pathogenicity:** It causes
 1. Wound infection following contact of wounds with seawater
 2. Septicaemia in persons with pre-existing hepatic disease, following the consumption of contaminated raw oysters

42

Chapter

Yersinia, Pasteurella and Francisella

■ How did *Yersinia* acquire its name? Mention its important species.

- The genus is named so after Alexander Yersin who discovered it as the plague bacillus
- Important species are *Y. pestis*, *Y. enterocolitica* and *Y. pseudotuberculosis*
- These are primary pathogens of rodents

■ Which species of *Yersinia* causes plague?

Important member among the three species of *Yersinia* (*Y. pestis*, *Y. enterocolitica*, *Y. pseudotuberculosis*) causing human infection is *Y. pestis*, which is causative agent of plague.

■ Mention the identifying morphological characteristics of *Y. pestis*.

Morphological Characteristics (Fig. 42.1)

- Gram-negative bacilli
- Short and plump, $1.5 \mu \times 0.7 \mu$, rounded ends with convex sides
- Nonmotile and nonsporing but capsulated
- Arrangement—singly or in short chains
- Methylene blue stain—**Safety-pin appearance**, i.e. two ends densely stained and central area clear
- Pleomorphism is common and enhanced in 3% NaCl-containing media

■ Mention the cultural characteristics of *Y. pestis*, including media and colony characters.

Cultural Characteristics

- Aerobe and facultative anaerobe
- Grows at 2° – 45° C. Optimum temperature 27° C and optimum pH 7.2
- Can grow on ordinary media-like nutrient agar

Media and Colony Characters

Media

- **Solid:** Nutrient agar, blood agar and MacConkey's agar
- **Liquid:** Ghee broth

Colonies

- **Nutrient agar:** Small, delicate, transparent colonies after 24–48 hours that increase in size and become opaque on further incubation

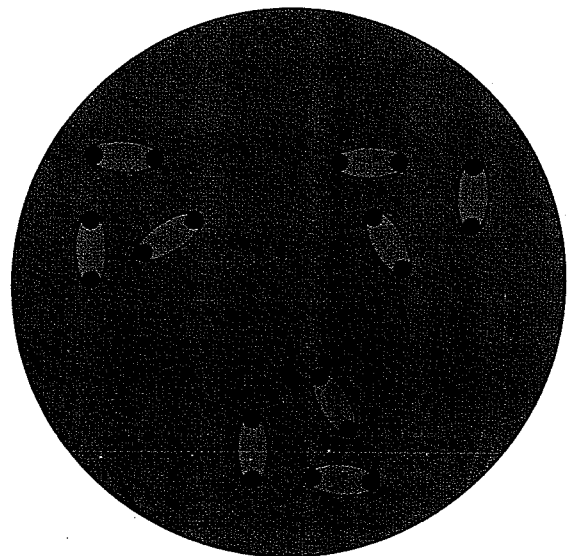


Fig. 42.1 *Yersinia pestis*—Safety-pin appearance.

- **Blood agar:** Dark brown colonies due to absorption of haemin pigment
- **MacConkey's agar:** Nonlactose fermenting colony (colourless)
- **Nutrient broth:** Flocculent growth at bottom and along sides of the tube
- **Ghee broth:** If grown in flask, it shows **stalactite growth**, (growth which hangs down into broth from surface)

■ Which biochemical reactions are characteristic of *Y. pestis*?

Biochemical reactions characteristic of *Y. pestis* are:

- Catalase—positive
- | | | | | | | | |
|---|---|---|---|---|----|----|---|
| L | G | M | S | I | MR | Vi | C |
| — | + | + | — | — | + | — | — |
- (+ = positive, — = negative)

■ Mention the effects of temperature, disinfectants and bacteriophage on survival of *Y. pestis*.

Effects of temperature, disinfectants and bacteriophage on *Yersinia* are:

- **Temperature:** It remains viable for months in cold environment. It is easily destroyed by heating and drying.
- **Disinfectants:** It is easily destroyed by disinfectants
- **Bacteriophage:** All strains are lysed by specific antiplague bacteriophage at 22°C

■ Describe the antigenic structure of *Y. pestis*.

Antigenic Structure

Y. pestis has complex Ag structure of 20 different Ags. Some of them are:

- F-I Ag (Fraction I Ag)—it is protein envelope Ag, inhibits phagocytosis and present only in virulent strains
- The V and W proteins—formed in virulent strains. These are considered as virulence factors as they inhibit phagocytosis and intracellular killing of bacteria

■ Mention the determinants of pathogenicity in *Y. pestis*.

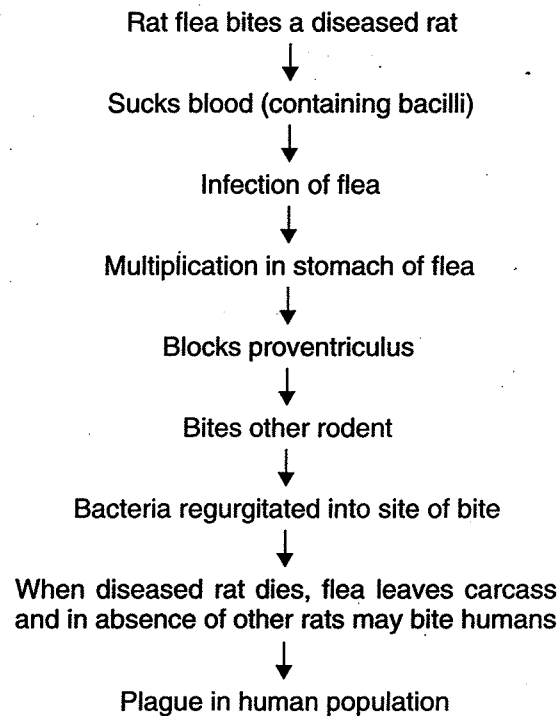
Determinants of pathogenicity are:

- V and W Ag
- F-I envelope Ag
- Virulence associated factors—pesticin, coagulase, fibrinolysin
- Ability to synthesize purine
- Production of pigmented colony on haemin-containing media

■ Describe the pathogenesis of *Y. pestis*. Draw an outline sketch showing how infection is transmitted from rodents to human beings.

Pathogenesis of *Y. pestis*

- *Y. pestis* causes a zoonotic disease plague, transmitted by bite of flea: *Xenopsylla cheopis*
- Flea—wingless insect has 2 hind legs with which it jumps from rat to rat or rat to man, cannot attain height more than 2 feet
- In humidity, they survive longer, in Indian climate for 47 days



Flowchart 42.1 Urban or domestic cycle of plague.

Two natural cycles of plague are:

1. Urban or domestic
2. Wild or sylvatic
 - Urban cycle involves man and rodents (rat; Flowchart 42.1)
 - Wild cycle involves wild rodents, e.g. squirrels, chipmunk, mice

■ **Describe the clinical features of plague.**

Plague occurs in three forms. These are:

1. **Bubonic**
 - After incubation period of 2–5 days draining lymph nodes are enlarged
 - As plague bacillus enters through bite on legs, inguinal lymph nodes are involved, hence it is called bubonic plague
 - Glands are enlarged, suppurate, bacilli enter in blood and produce septicaemia
2. **Septicaemic**
 - Bacteria in blood are denoted as septicaemic plague
 - Massive blood vessel involvement leads to haemorrhage in skin and mucosa and gangrene, therefore the disease was also called **Black Death**
3. **Pneumonic**
 - Bacterial emboli may be trapped in lungs causing pneumonic plague
 - Transmission can occur by air borne route (droplet infection)
 - May occur in epidemic form
 - It is characterized by fever, cough with expectoration, mucoid blood tinged sputum, chest pain, and difficulty in breathing. Towards the end-stage of the disease, patient develops cyanosis and circulatory failure and fatality rate is very high

■ Discuss the epidemiology of plague.

Epidemiology of Plague

- 1 It is endemic in many parts of India
- It is responsible for 41 epidemics before the birth of Christ and 109 in next 15 centuries worldwide
- 45 pandemics occurred between 1500 and 1700
- Quiescent in the 18th and 19th century
- Last pandemic occurred in 1894
- India remained free from plague from the year 1967–1993
- Outbreak reported in August–October 1994 in different states—Gujarat, Maharashtra, Delhi, Karnataka, Uttar Pradesh and Madhya Pradesh
- Bubonic plague occurred in 1994 in Beed (Maharashtra) and pneumonic plague outbreak in Surat

■ How is *Y. pestis* infection determined through laboratory diagnosis?

Steps involved in laboratory diagnosis of *Y. pestis* infection are:

Specimen

Bubo exudates, sputum and blood.

Collection

With all aseptic precautions bubo exudate is collected by hypodermic syringe.

Processing of Specimen

Microscopic Examination

- **Gram stain:** Short and plump Gram-negative bacilli with rounded ends, convex sides, arranged singly or in short chains
- **Methylene blue stain:** Bacilli show safety-pin appearance, i.e. two ends densely stained and central area clear

Culture

Specimen is inoculated on the following media:

- **Nutrient agar:** Small, delicate, transparent colonies, which increase in size and become opaque on further incubation
- **Blood agar:** Colonies are dark brown due to absorption of haemin pigment
- **MacConkey's agar:** Nonlactose fermenting colonies

Biochemical Reactions

- Catalase—positive

L	G	M	S	I	MR	Vi	C
---	---	---	---	---	----	----	---

–	+	+	–	–	+	–	–
---	---	---	---	---	---	---	---

(+ = positive, – = negative)

Animal Inoculation

Inoculated animal (rat) dies in 2–5 days; on necropsy exudates from different organs show bacilli on methylene blue staining.

Serology

Ab to FI Ag by agglutination, CFT and passive haemagglutination.

Laboratory Diagnosis in Animals

- Before examining the rats, which died of plague, following precautionary measures should be taken:
 - Pour kerosene over carcass to remove ectoparasites
 - Dip them in disinfectant, e.g. Lysol
- Samples from spleen, lymph nodes and blood collected and processed as in human plague

■ What can be done to prevent *Y. pestis* infection?

Prophylaxis

1. Control of fleas and rodents
2. Protection by vaccine in those who are occupationally exposed to risk
3. Chemoprophylaxis—tetracycline orally for 5 days

■ Mention the characteristic features and pathogenicity of *Yersinia pseudotuberculosis* and *Yersinia enterocolitica*.

Yersinia pseudotuberculosis

Characteristic features

It differs from *Y. pestis* in the following aspects:

- Poor growth on MacConkey's agar
- Motile at 22°C but not at 37°C
- Urease—Positive
- Failure to be lysed by antiplague bacteriophage

Pathogenicity

- **Pseudotuberculosis**—a zoonotic disease
- **In animals** (guinea pigs, rabbits, birds, etc.), infection occurs through alimentary tract resulting in *epizootic* and *enzootic* disease resembling tuberculosis, characterized by multiple nodules in liver, spleen and lungs as observed in tuberculosis, hence named as pseudotuberculosis
- **In humans**, infection is acquired through ingestion of materials contaminated with animal faeces, it may present as fatal typhoid-like illness with hepatosplenomegaly and purpura or mesenteric lymphadenitis or gastroenteritis

Laboratory diagnosis

- By isolation of the organism or by demonstrating Abs in patient serum

Yersinia enterocolitica

Characteristic features

- Gram-negative coccobacillus
- Motile at 22°C. Optimum temperature for growth is 22°C
- Grows on blood agar and MacConkey's agar—form pin-point, nonlactose-fermenting colonies
- Many strains are indole and VP positive

Pathogenicity

It produces 3 varieties of diseases in humans, viz.

- In younger children—self-limited disease
- In elder children—mesenteric adenitis and inflammatory ileitis
- In adults—systemic disease characterized by bacteraemia, meningitis, arthralgia, and erythema nodosum

Laboratory diagnosis

By isolation of the organism or by demonstrating Abs in patient serum.

■ **Enumerate the distinguishing features of *Pasteurella*.**

Distinguishing features of *Pasteurella* are:

- **Morphology**
 - Gram-negative bacillus
 - Nonmotile
- **Differs from *Yersinia* in being**
 - Oxidase-positive
 - Indole-positive
- **Species: *P. multocida*** – Primarily causes haemorrhagic septicaemia in animals
- **Pathogenicity:** No growth on MacConkey's agar
- **Infection in man:** It occurs the following animal bites or trauma
- **Clinical features:** Local suppuration at the site of animal bite in the form of wound infection, cellulitis, abscess, osteomyelitis
 - Meningitis following head injury
 - Respiratory tract infection
- **Treatment:** Tetracyclin and streptomycin

■ **Enumerate the distinguishing features of *Francisella tularensis*.**

Distinguishing features of *Francisella tularensis* are:

- It is a causative agent of **Tularaemia**—a disease of rabbit and other rodents
- Infection is transmitted by ticks and other arthropod vectors

Morphology

- Gram-negative bacilli
- Size: about $0.3\text{--}0.7\ \mu \times 0.2\ \mu$
- Capsulated but nonmotile

Cultural characters

It has fastidious growth requirements and grows on special media such as Francis blood dextrose cystine agar—forms minute transparent colonies after 3–5 days

Pathogenicity

- Infection in humans occurs by direct contact with animals, can also occur by ingestion of contaminated meat or water and inhalation
- **In humans, tularemia may present** as local ulceration with lymphadenitis, a typhoid-like fever with glandular enlargement or influenza-like respiratory infection

Laboratory diagnosis

By smear examination, culture or by animal inoculation in guinea pigs. Alternatively, by demonstrating agglutinating Abs in patient serum—a titre of 1:80 or more is considered as significant.

Treatment

Streptomycin is the drug of choice.

Prophylaxis

A live attenuated vaccine is given to persons with high risk of infection.

43

Chapter

Bordetella

■ Who discovered *Bordetella*?

Bordet and Gengou (1900) observed and cultivated the bacillus from a case of whooping cough.

■ Name the pertussis-causing species of *Bordetella*.

- Pertussis-causing species of *Bordetella* are:
 - *B. pertussis*
 - *B. parapertussis*
 - *B. bronchiseptica*
- *Bordetella pertussis* is the commonest cause of pertussis. It is also called Bordet and Gengou bacillus

■ Mention the characteristic morphological features of *B. pertussis*.

Morphological Features

- *B. pertussis* is a small Gram-negative coccobacillus
- Size: $1-1.5 \mu \times 0.3 \mu$
- In primary culture, it is of uniform size but may become filamentous on subculture
- It is nonmotile and nonsporing
- Fresh isolates are capsulated but tend to lose capsule on repeated subcultures
- Fresh isolates also have fimbriae

■ Mention the cultural characteristics of *B. pertussis*?

Cultural Characteristics

- It is an obligate aerobe grows slowly
- Its optimum temperature is $35^{\circ}-36^{\circ}\text{C}$

Media Used

Media used for isolation is Bordet-Gengou glycerine-potato-blood agar (starch and blood in medium help to neutralize toxic substances)

Colony Characters

After 72 hours of incubation—small, dome-shaped, smooth, opaque, viscid, gray-white, refractile, glistening colony giving “bisected pearls” or “mercury drops” appearance is formed

- Colonies are surrounded by hazy zone of haemolysis
- In culture films—their appearance is described as “thumb print appearance”
- Confluent growth—gives “aluminium paint” appearance

■ Mention the biochemical features of *B. pertussis*.**Biochemical Features**

- It is biochemically inactive, does not attack sugars
- Its indole, catalase and oxidase tests are positive

■ Comment on resistance/ sensitivity of *B. pertussis*.

- *B. pertussis* is a delicate organism, which is killed by heat at 55°C for 30 minutes, drying and disinfectants
- It may survive on glass for 5 days, on clothes for 3 days and on paper for few hours

■ Enumerate the determinants of virulence in *B. pertussis*.**Virulence determinants of *B. pertussis* are:**

- Heat labile toxin
- Lipopolysaccharides—endotoxin
- Tracheal cytotoxin
- Pertussis toxin
- Adenylate cyclase
- Haemolysin
- Filamentous haemagglutinin

Heat labile toxin

- It is a cytoplasmic protein, believed to cause vasoconstriction and inflammatory reaction
- Along with endotoxin, it causes rhinorrhoea, sneezing and mild cough in catarrhal stage of disease

Endotoxin (LPS)

It has pyrogenic effect and Shwartzman reactivity.

Tracheal cytotoxin

- This is probably responsible for destruction of ciliated respiratory epithelium resulting in accumulation of mucus, bacteria and inflammatory debris in lungs leading to severe cough
- The disruption of ciliary function leads to secondary bacterial infections

Pertussis toxin

- It is a lymphocytosis promoting histamine—the sensitizing factor
- It is responsible for paroxysmal cough of pertussis

Adenylate cyclase

It causes inhibition of chemotaxis, phagocytosis, phagosome—lysosomal fusion, super oxide generation and bactericidal activity of leucocytes, macrophages and monocytes.

Haemolysin

It is responsible for producing haemolysis on media.

Filamentous haemagglutinin

It is a cell surface protein, exists as filamentous rod, which mediates fusion of bacteria.

- **Discuss pathogenicity of *Bordetella* spp. Mention the clinical features of infections caused by them.**

Pathogenicity and Clinical Features

- Infection is confined to respiratory tract. It does not invade blood stream
- Obligate human parasites causing whooping cough are:
 - 95% cases caused by *B. pertussis*
 - 5% cases caused by *B. parapertussis*
 - Rarely by *B. bronchiseptica*
- Incubation period: 1–2 weeks, consists of three stages:
 1. Catarrhal
 2. Paroxysmal
 3. Convalescent

Each stage lasts for 2 weeks.

Catarrhal stage

It is characterized by low-grade fever and dry irritating cough. It is a stage of maximum infectivity

Paroxysmal stage

Cough increases in intensity and comes in bouts. During paroxysms, patient is subjected to violent spasm of continuous coughing followed by long inrush of air into almost empty lungs with characteristic whoop.

Convalescent stage

Frequency and severity of cough decreases.

Complications

1. Due to pressure effects of coughing
 - Sub-conjunctival haemorrhage
 - Emphysema
2. Respiratory—bronchopneumonia and collapse of lungs
3. Neurological—convulsions and coma

It may lead to permanent sequelae as epilepsy, paralysis, retardation and blindness or deafness.

- **Write in short the laboratory diagnosis of pertussis.**

Specimens

- Respiratory secretions
- Nasal swab
- Postnasal swab
- Nasopharyngeal aspiration

Collection

- **Cough plate method**—used for culture. Sample is collected by holding culture plate 10–15 cm in front of patient's mouth during bout of coughing, so that droplets impinge directly on medium
- **Postnasal swab**—West's postnasal swab bent at 40° angle is used to collect sample from posterior pharyngeal wall

- **Pernasal swab**—is passed along the floor of nasal cavity up to posterior wall of nasopharynx
- **Nasopharyngeal aspirates**—may also be collected through soft catheter attached to syringe

Transport

If delay in transport is unavoidable, then one of the following transport media can be used:

- Regan-Lowe semisolid medium
- Casamino acid solution
- Modified Stuart's medium

Processing of Specimen

Microscopic Examination

Immunofluorescence test helps to detect bacilli in smears.

Culture

- Sample inoculated on Bordet-Gengou glycerine-potato-blood agar
- Colonies after 48–72 hours are small, dome-shaped, smooth and opaque
- Identification is confirmed by microscopic examination and serology

Biochemical Reactions

Not important in identification.

Slide Agglutination

Used for serotyping.

Ab Detection

Paired sera is tested by:

- Agglutination
- Indirect haemagglutination
- Complement fixation test (CFT)
- Enzyme-linked immunosorbent assay (ELISA)

Nucleic Acid Detection

By polymerase chain reaction (PCR) from nasopharyngeal aspirate.

■ Which antibiotics are suggested for treating pertussis?

Antibiotics for treatment are:

- Erythromycin
- Tetracycline
- Chloramphenicol
- Amoxycillin
- Rifampicin
- Ciprofloxacin

■ Enumerate the details of a vaccine that is administered for preventing pertussis.

- **Prophylactic vaccine:** DPT vaccine
- **Type:** Killed vaccine

- **Route:** Deep intramuscular
- **Schedule:**
 - 3 doses at 4-weeks interval from 1 to 1½ months of age
 - 1st booster DT—1½ years of age
 - 2nd booster DT—5–6 years
- **Adverse reactions:** Erythema, local swelling and pyrexia

44

Chapter

Brucella

■ Describe the causal agent of brucellosis. Who isolated the genus first?

- *Brucella* spp., are small Gram-negative coccobacilli cause the zoonotic disease brucellosis, primarily affecting goats, sheep, cattle, buffaloes, pigs and other animals.
- David Bruce (1887) isolated them first

■ Name the medically important species of *Brucella*.

Species of medical importance are:

- *B. melitensis*
- *B. abortus*
- *B. suis*
- *B. canis*

■ Enumerate the characteristic morphological features of *Brucella*.

Morphological Features

- Small Gram-negative coccobacilli
- Size: $0.5-0.7 \mu \times 0.6-1.5 \mu$
- Nonmotile, noncapsulated and nonsporing

■ Describe cultural characteristics of *Brucella*; include media and colony characters.

Cultural Characteristics

- They are strict aerobes, some species are capnophilic requiring 5–10% CO₂
- Optimum temperature for growth is 37°C (range, 20°–40°C), pH favourable for growth is 6.6–7.4
- They can grow on ordinary media but growth is slow, growth is improved by addition of blood and serum

Media Used

- Serum dextrose agar
- Serum potato infusion agar
- Trypticase soy agar
- Tryptose agar
 - Addition of bacitracin, polymyxin and cycloheximide makes the medium selective

Colony Characters

- On **Serum dextrose agar** it shows small, 1 mm in diameter, low convex, circular, smooth, transparent and glistening colonies
- On **MacConkey's medium** it shows nonlactose-fermenting colonies

■ **Mention the conditions that are adverse to survival of *Brucella*.**

- They are destroyed by heat at 60°C in 10 minutes
- Also destroyed by pasteurisation of milk
- Sensitive to acid, hence die in butter and cheese due to lactose fermentation
- Survive for 6–10 weeks in dust and soil

■ **Mention the biochemical reactions of *Brucella*.**

Biochemical Reactions

- They utilize carbohydrates oxidatively
- Catalase—Positive
- Urease—Positive
- Oxidase—Positive
- Reduce nitrates
- Indole, methyl red, Voges-Proskauer and citrate tests—Negative

■ **On what basis is *Brucella* differentiated into species? Name the species.**

On the basis of CO₂ requirement, urease, H₂S production and agglutination by monospecific sera they are divided into 6 species. The six species are:

1. *B. melitensis*
2. *B. abortus*
3. *B. suis*
4. *B. canis*
5. *B. ovis*
6. *B. neotome*

■ **Describe the antigenic structure of *Brucella*.**

- Somatic Ag of *Brucella* has 2 antigenic determinants—A and M
 - *B. abortus* has 20 times A as M
 - *B. melitensis* has 20 times M as A
 - *B. suis* shows intermediate pattern
- This feature is important in species identification by agglutination

■ **Which disease is caused by *Brucella*? Name the pathogenic species.**

- *Brucella* causes brucellosis, which is also known as Mediterranean fever, Malta fever and undulant fever
- *B. melitensis* is most pathogenic, *B. abortus* and *B. suis* are of intermediate pathogenicity

■ **How is brucellosis transmitted to human beings?**

- **Brucellosis is a zoonotic disease:** Infected animal sheds bacteria through vaginal secretions, urine and milk
- **Route of entry:** Infection is transmitted to humans in the following ways:
 - Direct contact with animal tissue as in dairy workers, farmers, veterinarians
 - Ingestion of contaminated meat, raw vegetables and infected milk
 - Inhalation of aerosolized organisms
 - Accidental injection as in laboratory workers

■ **Describe the pathogenesis of brucellosis.**

- *Brucella* is an intracellular pathogen mainly affecting reticuloendothelial system. This accounts for their refractoriness to treatment
- The route of disease development is presented in Flowchart 44.1

■ Mention the clinical features of brucellosis.

Brucellosis is of the following three types:

1. Latent infection
2. Acute infection
3. Chronic brucellosis

Latent Infection

Patient is serologically positive but clinical features are not seen.

Acute Infection

Prolonged bacteraemia, irregular fever with chills, malaise, night sweat, myalgia, arthralgia, lymphadenopathy and hepatosplenomegaly.

Chronic Brucellosis

It is nonbacteraemic, low-grade infection with periodic exacerbation, lasts for years.

■ Describe the laboratory diagnosis of *Brucella* infections.

Specimens

- Blood is the commonest specimen
- Bone marrow and lymph nodes can be used and rarely urine, sputum, CSF, milk, vaginal discharge and seminal fluid are used

Collection

5–10 ml of blood collected by venepuncture in trypticase soy broth.

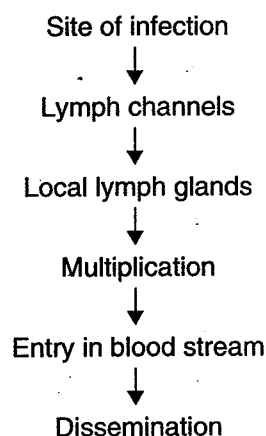
Blood Culture

The following three types of culture methods can be used:

1. **Trypticase soy broth** is inoculated with specimen and subcultures are made on serum dextrose agar (SDA) from blood culture bottle every 3–5 days
2. **Castaneda's method** - This method is used for rapid detection of causative agents
 - Castaneda's medium is a diphasic medium containing both liquid and solid media in the same bottle. The specimen is inoculated into liquid medium and bottle is incubated in upright position. For inoculation of agar, bottle is tilted at intervals so that the broth flows over the agar slant. Again it is incubated in upright position. The colonies formed on agar slant are studied and identified (Fig. 44.1)
 - Advantages:
 - It minimizes the materials and manipulations required for processing of specimen
 - It reduces chances of contamination
 - It reduces risk of infection to laboratory workers
3. **BACTEC** is a rapid and automated system for culture, gives results in 5–6 days

Serology

- Serological tests detect IgM and IgG
- They appear 7–10 days after clinical infection
- As disease progresses, IgM declines and IgG persists in rising titres
- In chronic infections, IgM are absent and IgG are present



Flowchart 44.1 Development of brucellosis.

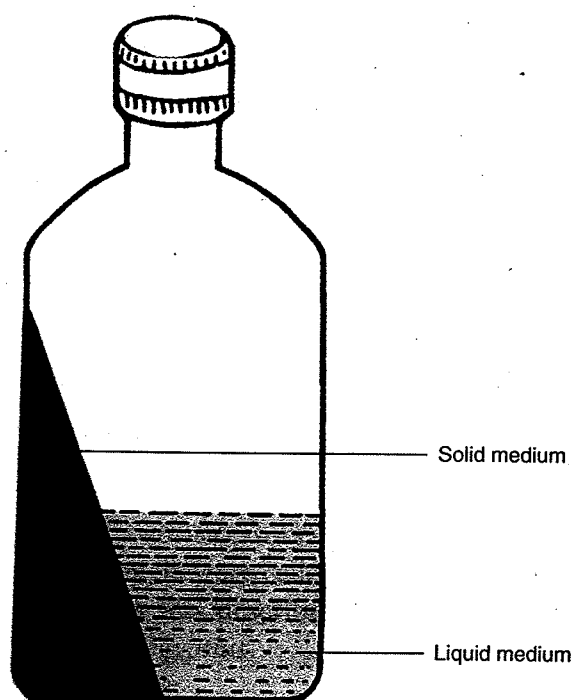


Fig. 44.1 Castaneda's medium.

- **Agglutination test**
 - It detects IgM, whereas IgG and IgA act as blocking antibodies
 - Tube agglutination test: Equal volumes of serial dilutions of serum mixed with standard antigen and incubated for 24 hours at 37°C and observed for agglutination
 - Titre of 1:160 or more is considered significant in a single test
 - False negative—sera with blocking antibodies and prozone phenomenon
 - False positive—cholera, tularemia and Yersinia infection
- **Complement fixation test (CFT):** It detects both IgM and IgG
- **Enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA):** These can detect IgG, IgM and IgA separately

Skin Test–Brucellin Test

- Ag is injected on forearm and observed for reaction after 24 hours. Positive reaction is in the form of raised, erythematous plaque 2–6 cm in diameter
- It is used to detect delayed hypersensitivity to *Brucella* antigen
- It is not useful for diagnosis of infection. It indicates past exposure to *Brucella* infection

■ How is brucellosis diagnosed in animals?

- Methods used for diagnosis in humans can also be used for diagnosis in animals
- Some rapid tests are:
 - Rapid plate agglutination
 - Rose Bengal card test
 - Milk ring test
 - Whey agglutination test

Milk Ring Test

- It is used for detection of infected animals in dairies by using pooled milk sample
- Whole milk is mixed with drop of stained brucella Ag and incubated in water bath at 70°C for 40–50 minutes. If Abs are present in milk, the bacilli are agglutinated and rise with cream

to form blue ring at top leaving milk unstained. If Abs are absent, ring is not formed and milk remains uniform blue

- This is most commonly used test as it is simple, and uses milk sample and not serum

■ How can brucellosis infection be prevented?

- Contraction of brucellosis can be prevented through vaccination. However, vaccine is available for cattle but not for humans
- Pasteurisation of milk
- Detection and elimination of infected animals

■ How can brucellosis be treated?

Brucellosis can be treated with combination of streptomycin with tetracycline or rifampicin with doxycycline

45

Chapter

Haemophilus

■ Write down the general concepts related with *Haemophilus*.

- The genus *Haemophilus* contains nonmotile, nonsporing, often pleomorphic, Gram-negative coccobacilli
- They require one or both of two accessory growth factors (X and V) present in blood for their growth and that is why they are called *Haemophilus* (meaning blood loving)
- The first member of this genus was described by Robert Koch in conjunctival exudates in Egypt and it is known as *H. aegyptius*
- Species belonging to this genus are:
 - *H. influenzae* (*influenza bacillus*/Pfeiffer's bacillus) - Major pathogen in the genus
 - *H. ducreyi*
 - *H. parainfluenzae*
 - *H. aphrophilus*
 - *H. paraphrophilus*

■ What are the morphological characteristics of *H. influenzae*?

Morphological Characteristics

- Gram-negative, slender and short coccobacilli
- Size: $1-2 \mu \times 0.3-0.5 \mu$
- Pleomorphic - filamentous forms are seen in cerebrospinal fluid
- Nonmotile and nonsporing (Fig. 45.1)
- Few strains have capsule, which can be demonstrated by
 - India ink wet films
 - Quellung reaction

■ Describe the cultural features of *H. influenzae*, including media and colony characters.

Cultural Features

- Growth is better in aerobic condition - needs 5–10% CO₂ for growth
- Temperature range for growth is 20°–42°C. Optimum temperature 35°–37°C
- Growth requirements—fastidious
- Requires factor X and V-bacterial vitamins present in blood for growth
- X-factor
 - It is a heat stable iron porphyrin—haematin present in blood

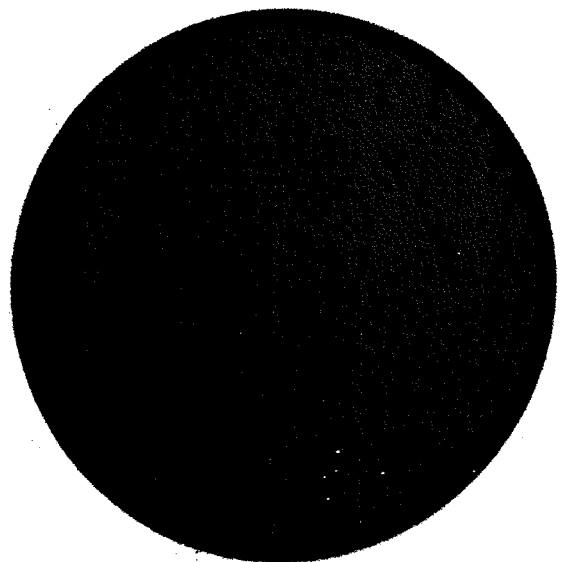


Fig. 45.1 *Haemophilus influenzae*.

- It is necessary for synthesis of enzymes involved in respiration such as catalase, peroxidase and cytochrome oxidase
- **V-factor**
 - It is a heat labile coenzyme—nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP)
 - It is necessary for oxidation-reduction process in a growing bacterial cell
 - It is present in blood and also synthesized by some bacterial species such as *Staph. aureus*

Media and Colony Characters

- Sheep blood agar—not suitable for growth where *H. influenzae* forms pinpoint colonies because
 - X- and V-factors are not liberated from red blood cells as there is no lysis of red cells
- **Blood agar with streak of *Staph. aureus***—shows satellitism
 - It is used because staphylococcal cells produce V-factor, *Staph. aureus* causes lysis of RBC and release of X-factor
- **Chocolate agar**—it is good for isolation because X and V-factors are released from RBCs by lysis
 - Heating also results in inactivation of NADase enzyme that hydrolyses V-factor
- **Levinthal's agar**—it is a transparent medium containing extract of blood
- **Filde's agar**—it is composed of Nutrient agar and digest of blood
- On chocolate, Levinthal's and Filde's agar—colonies are gray, transparent, smooth and low convex

■ What is satellitism? Discuss how this phenomenon is used in the identification of *H. influenzae*.

Satellitism (Fig. 45.2)

- It is a test used for identification of *H. influenzae* from clinical specimens
- When plate of blood agar is streaked across with *Staph. aureus* and inoculated with specimen containing *H. influenzae*, colonies of *H. influenzae* will be large and well developed alongside the streak of *Staph. aureus* and become smaller as distance increases. It is called satellite growth of *H. influenzae* and phenomenon is called satellitism

Procedure for Identification

- Specimen containing *H. influenzae* is inoculated on blood agar
- *Staph. aureus* is streaked across the plate
- Plate is incubated overnight and observed for colonies

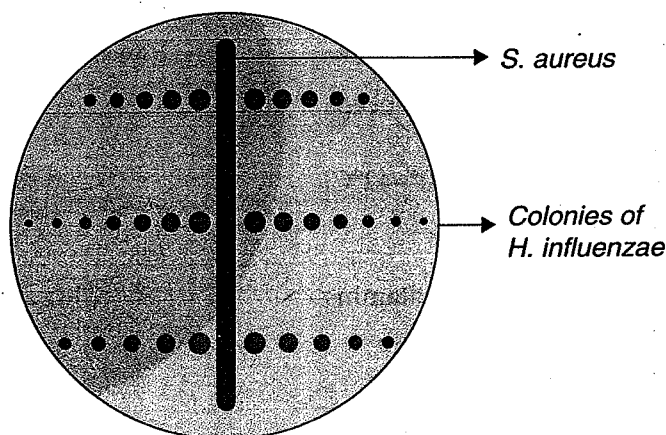


Fig. 45.2 Satellitism.

Interpretation

Colonies near streak of *Staph. aureus* are larger and become smaller as distance increases, indicating satellitism.

Mechanism

Lysed RBCs in the agar surrounding *Staph. aureus* provide X-factor, and staphylococcal cells themselves secrete V-factor during growth, hence colonies near streaks are larger and become smaller as distance increases.

Importance of Satellitism

It shows that X and V-factors are essential for growth of *H. influenzae*.

■ Mention the biochemical reactions of *H. influenzae*.

- Biochemical reactions that characterize *H. influenzae* are:
 - Catalase—positive
 - Oxidase—positive
 - Nitrate reduction—positive
- On the basis of indole, urease and ornithine decarboxylase activity, it is divided into eight biotypes—groups I–VIII
- Majority of clinical isolates belong to Groups I–III and Type b organisms belong to biotype I

■ Describe the antigenic structure of *H. influenzae*.

Some strains of *H. influenzae* have capsular polysaccharide antigens while others have outer membrane protein and lipo-oligosaccharide antigens.

Capsular Polysaccharide

- On the basis of this Ag, Pittman divided *H. influenzae* into six types—a, b, c, d, e and f
- Most infections are caused by serotype b
- Methods used for typing are:
 - Quellung reaction
 - Agglutination
 - ELISA
 - Countercurrent immunoelectrophoresis (CIEP)
- Capsular Ag induces IgG, IgM and IgA Abs, which are protective, hence used in vaccine preparation

Outer membrane protein antigen - *H. influenzae* type b is classified into 13 subtypes.

■ In what ways can *H. influenzae* be destroyed?

- *H. influenzae* is a delicate organism
- It is inactivated by moist heat at 55°C in 30 minutes, refrigeration at 4°C, drying and disinfectants
- It dies in 1–48 hours in airborne droplets

■ Which factors contribute to virulence of *H. influenzae* and how?

The factors governing virulence are as follows:

- Capsular polysaccharide—resists phagocytosis. Loss of capsule is associated with loss of virulence
- Pili—help in attachment

- Outer membrane protein—contributes to adhesion and invasion
- IgA protease—Cleaves IgA-1

■ Discuss the pathogenicity of *H. influenzae*.

- *H. influenzae* is an exclusively human pathogen
- The diseases caused by it can be divided into the following two groups:
 1. Invasive
 2. Noninvasive

Invasive

- Meningitis
- Laryngoepiglottitis
- Pneumonia
- Bacteraemia

Meningitis

- Disease is more common in children between two months and three years of age
- Fatality rate is up to 90% in untreated cases
- Bacilli reach meninges from nasopharynx through bloodstream

Laryngoepiglottitis or croup

- Second common disease
- Acute inflammation of epiglottis with obstructive laryngitis, which may need tracheostomy
- Untreated cases may be fatal in hours
- Usually associated with bacteraemia

Pneumonia

- Common in infants
- In adults and children, it causes lobar pneumonia
- Bronchopneumonia may occur as secondary infection with noncapsulated strains

Suppurative infections

- Arthritis, endocarditis, pericarditis may develop due to haematogenous dissemination
- Buccal periorbital cellulitis

Noninvasive

- Otitis media
- Sinusitis
- Acute exacerbation of chronic bronchitis

Otitis media, sinusitis

Secondary to viral infection.

Chronic bronchitis or bronchiectasis

It is associated with Pneumococci in acute exacerbation of chronic bronchitis.

■ Differentiate between invasive and noninvasive infections of *H. influenzae*.

Features distinguishing invasive infections from noninvasive infections are presented in Table 45.1.

Table 45.1 Differences between invasive and noninvasive infections

Invasive infections	Noninvasive infections
1. Bacilli are primary pathogens in these infections	Bacilli are secondary invaders
2. Examples: Meningitis, laryngoepiglottitis, pneumonia, bacteraemia	Examples: Otitis media, sinusitis, acute exacerbation of chronic bronchitis
3. Usually found in children	Usually seen in adults
4. Caused by capsulated strain usually type 'b'	Caused by noncapsulated strains
5. Spread via haematogenous route	Spread – local spreading

■ Describe the laboratory diagnosis of *H. influenzae* infections.

Specimens

Cerebrospinal fluid, blood, sputum, throat swab, pus, aspirate from joints and sinuses.

Collection

- Cerebrospinal fluid—in dry sterile container by lumbar puncture
- Blood—it can be collected in nutrient broth as it will be enriched with X and V-factors from blood sample
- Sputum—in a dry, leak-proof, sterile container

Transport

- As it is a delicate bacterium, it should be plated immediately and should never be refrigerated.

Microscopic Examination

It includes the following methods:

- **Wet preparation**—of cerebrospinal fluid shows plenty of pus cells and bacteria
- **Gram stain**—shows slender Gram-negative coccobacilli, pleomorphic-filamentous forms seen in cerebrospinal fluid
- **Immunofluorescence test**—direct detection of bacilli in smears by treating with antibody conjugated with fluorescent tag
- **Quellung reaction**—direct demonstration of capsule of *H. influenzae* after mixing with antisera

Culture

- Blood agar with streak of *Staph. aureus* and chocolate agar are used
- Blood agar shows satellitism

Serotyping

Capsular serotypes can be detected by treating them with antisera.

Antibiotic Susceptibility Testing

By standard Kirby–Bauer disc diffusion method on haemophilus test medium using ampicillin, co-amoxyclov, chloramphenicol, cefotaxime, ceftazidime, cefuroxime, quinolones, etc.

■ By which methods can type 'b' antigens be detected?

Type 'b' antigen in urine, serum or cerebrospinal fluid can be detected by

- Counter immunoelectrophoresis
- Latex agglutination

- Coagglutination
- Enzyme-linked immunosorbent assay (ELISA)

■ **Name the antibiotics of choice used in the treatment of *H. influenzae* infections.**

Cefotaxime and ceftazidime are the antibiotics of choice for treatment of meningitis.

■ **How can spread of *H. influenzae* infections be prevented?**

- Immunity is type specific. As majority of infections are caused by type 'b', active immunization with "Hib PRP" vaccine is indicated

■ **Mention the characteristic features of *H. ducreyi*.**

H. ducreyi was first demonstrated by Ducrey.

Morphological Characteristics

- Gram-negative bacillus - shows bipolar staining
- Size: $1.5 \mu \times 0.6 \mu$
- Arranged in parallel chains or groups or whorls giving school of red fish or rail road track appearance

Cultural Characteristics

- Growth is poor on most media
- Requires X but not V-factor for growth
- Growth is improved by CO₂ supplementation and humid atmosphere
- Media used are:
 - 30% rabbit blood agar
 - Chocolate agar with isovitalect, fetal calf serum and vancomycin
 - Human blood clot
- Colony—pinpoint, translucent and gray

■ **Which disease is caused by *H. ducreyi*?**

- *H. ducreyi* causes chancroid—a highly contagious venereal disease causing single or multiple tender, nonindurated ulcers on genital area and enlarged and painful regional lymph nodes
- It is also called soft sore

■ **What laboratory diagnostic methods are used for identifying *H. ducreyi*?**

- Scraping from edge of ulcer or aspirate from lymph nodes is used for Gram staining and culture
- Further identification is done by agglutination with antiserum

■ **Which antibiotics are advisable for treating *H. ducreyi* infections?**

Sulphonamides, erythromycin, tetracycline, ciprofloxacin and cefotaxime can be used for treating *H. ducreyi* infections.

■ **Write in short about *H. aegyptius*.**

- *H. aegyptius* causes acute conjunctivitis (pink eye)
- Diagnosis can be made by microscopic examination and growth on chocolate agar

46

Chapter

Mycobacteria I: *Mycobacterium* *Tuberculosis*

■ **What are mycobacteria? Who isolated *Mycobacterium tuberculosis* and when?**

- Mycobacteria are acid-fast rods
- They also show filamentous forms like fungal mycelium, hence called mycobacteria
- Robert Koch isolated *Mycobacterium tuberculosis* in 1882

■ **Give the name of the three groups in which Mycobacteria are classified? Cite examples.**

Mycobacteria are classified into the following three groups:

1. Obligate pathogens
2. Opportunistic pathogens
3. Saprophytes

Obligate Pathogens

- *M. tuberculosis* complex: *M. tuberculosis*, *M. bovis*, *M. africanum* - cause tuberculosis.
- *M. leprae*—causes leprosy

Opportunistic Pathogens

- These are atypical mycobacteria or MOTT, e.g. *M. kansasii*, *M. avium*, and many others
- They cause diseases like tuberculosis

Saprophytic Mycobacteria - are nonpathogenic

- Some examples are:
 - *M. smegmatis*
 - *M. phlei*
 - *M. stercoris*
 - *M. butyricum*

■ **Mention the distinguishing morphological features of *Mycobacterium tuberculosis*.**

Morphological Features

- Acid-fast rods—of size about $2-3 \mu \times 0.4 \mu$
- Straight or slightly curved with rounded ends
- Branching and filamentous forms also seen
- Beaded, barred forms—seen especially in sputum
- Arranged singly or in small clumps (see Fig. 3.5—Acid fast bacilli)
- Nonmotile, nonsporing and noncapsulated
- *M. bovis* is more straight, stout and short as compared to *M. tuberculosis*
- Acid fastness—It is due to presence of mycolic acid around the cell

■ Which stains are useful in identifying *M. tuberculosis*?

Stains used for demonstration of *M. tuberculosis* are:

- **Z-N stain:** Bacilli appear red with blue background
- **Fluorescent stain:** Smear stained with auramine O or rhodamine and observed under fluorescent microscope. Tubercle bacilli appear as yellow luminous rods in a dark field

■ Enumerate the cultural characteristics of Mycobacteria. Which culture media are suitable for their growth and how do colonies appear on being cultured?

Cultural Characteristics

- *M. tuberculosis*—an obligate aerobe, *M. bovis* is microaerophilic
- Optimum temperature 37°C (range, 20°–40°C), optimum pH 7.0 (range, 6.0–7.0)
- Slow growing—takes 2–8 weeks to grow on medium, generation time—14–15 hours
- Grows well on enriched media containing serum, potato, blood and egg

Types of Culture Media

- The following two types of media are in use:
 1. **Solid media**
 - Containing egg - Lowenstein-Jensen medium, Dorset egg medium, Petragnani medium
 - Containing blood—Tarshis medium
 - Containing potato—Pawlosky's medium
 - Containing serum—Loeffler's serum slope
 2. **Liquid media**
 - Dubo's media, Middlebrook media, Proskauer media
- Solid media are usually used for cultivation and liquid for sensitivity testing, biochemical reactions, and Ag and vaccine preparation
- Amongst all solid media Lowenstein-Jensen (LJ) medium is commonly used and also recommended by the International Union Against Tuberculosis
- Middlebrook 7H10 and 7H12 can be used
 - **Advantage:** They are transparent allow early detection of colony

Colony Appearance

M. tuberculosis

- Colony is dry, rough, raised, wrinkled, irregular (5-r), also described as rough, buff and tough (Fig. 46.1)
- It grows luxuriantly in culture and growth is described as **eugonic growth**
- Growth is improved with addition of 0.5% glycerol
- It does not grow on medium with P-nitrobenzoic acid
- In liquid media, growth occurs as bottom creeps up the sides, forms prominent surface pellicle, which extends along sides above the medium

M. bovis

- Grows sparsely on L-J giving **dysonic growth**
- Colonies are small, smooth, moist breakup easily
- Media with 0.5% glycerol may inhibit its growth

■ Which new technique is employed for culturing mycobacteria and why?

- For rapid growth of mycobacteria **BACTEC system** is used
- It is more sensitive than routine culture



Fig. 46.1 *M. tuberculosis* colonies on L-J medium.

- It uses special broth (liquid media) with radiometric growth detection
- Usually ^{14}C labelled substrate is used and $^{14}\text{CO}_2$ evolved due to bacterial metabolism is measured

■ **List the biochemical tests that are used for detecting mycobacteria. Explain each reaction.**

Biochemical tests for detecting mycobacteria are as follows:

1. Niacin test
2. Neutral red test
3. Aryl sulphatase test
4. Nitrate reduction test
5. Amidase test
6. Catalase and peroxidase test
7. Susceptibility to pyrazinamide
8. Tween 80 hydrolysis
9. Susceptibility to TCH (thiophen 2-carboxylic acid hydrazide)

Niacin Test

- Mycobacteria when grown on egg medium form niacin, but most species have enzymes that convert free niacin-to-niacin ribonucleotide

- When 10% cyanogen bromide and 4% alcoholic aniline are added to suspension of culture, yellow colour develops within few minutes indicating positive test
- The test is positive in human type (*M. tuberculosis*) and negative in bovine type (*M. bovis*) of mycobacteria.

Neutral Red Test

- Virulent strain binds neutral red in alkaline buffer solution, but avirulent cannot
- Growth is added to ethyl alcohol
- After incubating for 1 hour, the supernatant, i.e. alcohol, is removed with pipette and neutral red is added to make fluid alkaline

After incubation, pink or red stained colonies are seen suspended in the fluid, indicating positive test.

Aryl Sulphatase Test

- Organism is cultured on a broth containing tri-potassium salt of phenolphthalein disulphate
- Activity of the enzyme aryl sulphatase is detected by release of phenolphthalein, which becomes red in alkaline conditions

Test is negative for *M. tuberculosis* and positive for atypical mycobacteria.

Nitrate Reduction Test

- Test organisms are incubated in nitrate broth. After 4 hours, broth is tested for reduction of nitrate to nitrite by addition of sulphanilic acid reagent
- If nitrites are present, pink or red compound is formed. When they are not detected, to test whether they are reduced beyond nitrite, zinc dust is added, zinc dust converts nitrate to nitrite. If no nitrites are detected even with addition of zinc dust, it is assumed that nitrates are reduced beyond nitrites by bacteria
- Test is positive (red colour) for *M. tuberculosis* and negative for *M. bovis*.

Amidase Test

- A solution of amide is incubated along with bacterial suspension. Then MnSO_4 , phenol and hypochlorite are added and tubes are placed in boiling water. Blue colour indicates positive test
- It is positive for *M. tuberculosis* when nicotinamide and pyrazinamide are used, acetamide, benzamide, carbamide can also be used in the test to differentiate atypical mycobacteria

Catalase and Peroxidase Test

- Equal volumes of 30% hydrogen peroxide and 0.2% catechol in distilled water are added in a test tube containing 5 ml of test culture and allowed to stand for few minutes
- Reaction is observed for appearance of bubbles for 1 minute and noted as positive
- Lack of bubbles indicate negative test. Browning indicates peroxidase activity

Most atypical mycobacteria are strongly catalase positive (show immediate frothing) and *M. tuberculosis* is weakly positive (shows slow release of bubbles one by one) and *M. tuberculosis* is peroxidase positive but atypical is negative.

Susceptibility to Pyrazinamide

M. tuberculosis is sensitive to 50 $\mu\text{g/ml}$ of pyrazinamide while other mycobacteria are resistant.

Tween 80 Hydrolysis

- Some mycobacteria possess lipase, which splits Tween 80 to oleic acid and polyoxyethylated sorbitol, which changes test solution from straw yellow to pink indicating Tween 80 hydrolysis
 - *M. bovis* is always negative
 - *M. tuberculosis* gives variable results
 - Some atypical mycobacteria give positive results

Susceptibility to TCH (thiophen 2-carboxylic acid hydrazide)

- *M. bovis* is susceptible to 10 µg/ml of TCH while *M. tuberculosis* is resistant.

■ How long can mycobacteria remain viable? How can they be destroyed?

- Mycobacteria remain viable in sputum for 20–30 hours, in droplet nuclei for 8–10 days, in cultures for 6–8 months
- They are relatively resistant to disinfectants
- They are sensitive to formaldehyde and glutaraldehyde
- Bacilli are killed in 2 hours after exposure to direct sunlight
- Ethanol is a good disinfectant, kills bacilli in 2–10 minutes

■ Mention the types of antigens present in *Mycobacterium* spp.

- Types of antigens present in *Mycobacterium* spp. are:
 - Group-specific polysaccharide antigen
 - Type-specific protein antigen
- Various protein Ags and polysaccharide Ag are used in serological tests to detect antibodies

■ Trace the route of disease development in cases of infections by *M. tuberculosis* and *M. bovis*.

- The course of development of disease in infections by *M. tuberculosis* and *M. bovis* are presented in Flowchart 46.1.

■ Mention the types of tuberculosis based on the organ-system affected.

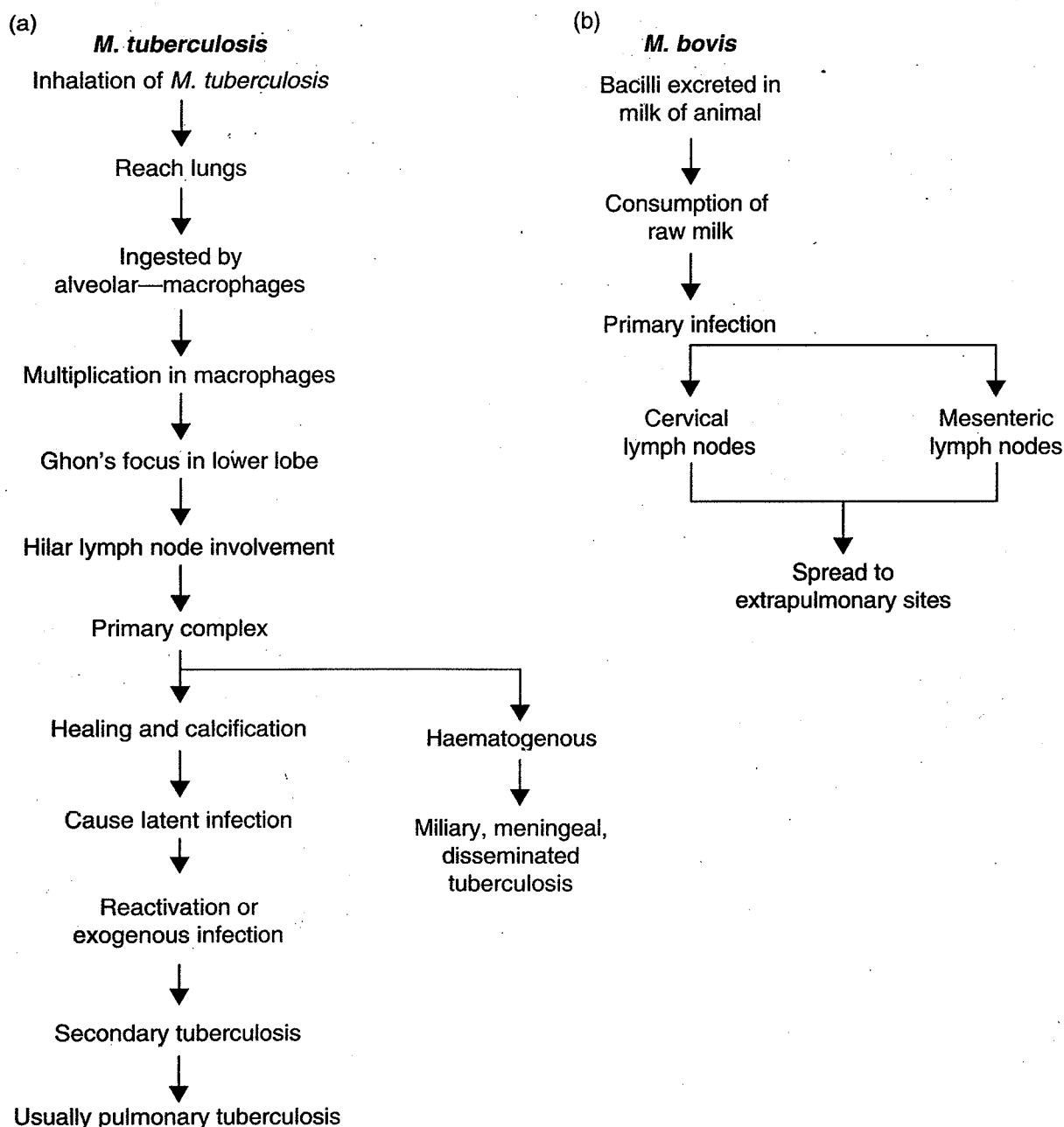
M. tuberculosis infection can involve various systems causing

- Pulmonary tuberculosis
- Renal tuberculosis
- Tubercular meningitis
- Bone and joint tuberculosis
- Miliary tuberculosis
- Intestinal tuberculosis
- Skin tuberculosis

■ List the clinical features of *M. tuberculosis* infection.

Clinical features of infections caused by *M. tuberculosis* are:

- Fever—evening rise of temperature
- Cough with expectoration
- Haemoptysis
- Weight loss
- Loss of appetite
- Signs of pleural effusion/consolidation/cavity



Flowchart 46.1 Disease development in (a) *Mycobacterium tuberculosis* and (b) *Mycobacterium bovis* infections.

■ **Discuss in detail the laboratory diagnosis of *Mycobacterium* infections.**

Specimens

- Sputum, laryngeal swab, bronchial washings and gastric lavage
- In meningitis—CSF (3–4 ml)
- In renal tuberculosis—urine (40 ml)
- Other samples collected—depending on site involved

Collection

- Sputum—early morning sample is collected. Sampling on three successive days increases chances of isolation. If sputum is scanty, laryngeal swab or bronchial washings are collected
- Gastric lavage is usually collected in children who swallow sputum
- Samples are collected using usual standard procedures (refer to Chapter 28 for details)

Transport

- If delay of more than 2 hours is expected, sample should be refrigerated. Refrigeration slows down multiplication of commensals
- If more delay is expected equal amount of cetylpyridium chloride-NaCl solution is added to sputum. It liquefies sputum and prevents growth of other bacteria and also maintains viability up to 8 days
- Pleural fluid should be collected in citrate bulb

■ **Mention the microscopic methods used for detecting mycobacteria.**

Preparation for Microscopic Examination

- Blood tinged or purulent part of sputum is used for preparation of smears
- Liquid specimens such as CSF, pleural fluid and urine should be centrifuged and sediment is used for preparation of smears
- Sensitivity of microscopy is 10,000 bacilli/ml of sputum

Methods for Demonstration

- **Zeihl-Neelsen stain:** It shows acid-fast bacilli (AFB) against blue/green/yellow background
- **Fluorescent stain:** It shows yellow luminous rods against dark background
- Minimum 300 oil immersion fields (OIF) of Z-N-stained smears are examined and reported with grading as:
 - 00 AFB in 300 OIF—AFB not seen
 - 1-2 AFB in 300 OIF—doubtful—repeat smear
 - 1-9 AFB in 100 OIF—1+
 - 1-9 AFB in 10 OIF—2+
 - 1-9 AFB in 1 OIF—3+
 - 10 or more AFB in 1 OIF—4+
- **Light Emitting Diode (LED) Fluorescent microscopy** - It is a newer, more sensitive and equally specific method

Concentration of Specimens

When AFB are not detected by direct microscopy then specimens are concentrated by different techniques. They can be classified into two types:

1. Methods that kill bacteria

- (a) Treatment with hypochlorites
- (b) Treatment with antiformin
- (c) Treatment with tergitol
- (d) Autoclave method—they kill bacilli so cannot be used for culture

2. Methods that do not kill bacteria

- (a) Petroff's method
- (b) Modified Petroff's method

These methods use alkali homogenization, instead of alkali—treatment with dilute acids such as 3% hydrochloric acid, 5% oxalic acid, cetrimide, zephiran can be used for concentration.

Petroff's Method

Equal volumes of sputum and 4% sodium hydroxide are mixed, incubated at 37°C for 20-30 minutes with intermittent shaking. Then centrifuged at 3000 rpm for 30 minutes and sediment used for microscopy and cultures.

■ How is culture technique used for identifying *Mycobacterium* spp.?

The culture technique used for identifying *Mycobacterium* spp. is described below:

Procedure

- Culture is a more sensitive technique than microscopy. It can detect 10–100 bacilli/ml of sputum
- Solid media - Concentrated sputum is inoculated on 2 L–J slants and incubated at 37°C
 - Cultures examined weekly up to 12 weeks before declaring negative
 - The slant is observed for dry, rough, raised wrinkled, irregular, colonies of *M. tuberculosis*, which are further identified by biochemical reactions
- Liquid media - Culture can be done by
 1. BACTEC method - gives result earlier.
 2. MGIT Mycobacteria growth indicator tube - consists of liquid modified Middlebrook 7H9 broth base medium known to yield better recovery and faster growth of mycobacteria.
 3. MODS (Microscopic Observation Drug Susceptibility) assay - a liquid culture-based method. Drug-free and drug-containing media are inoculated with specimens and cultures are microscopically examined. It can assess susceptibility to isoniazid and rifampicin.

Identification

To identify the isolates following observations are helpful:

- Slow growth at 36°C taking 2–6 weeks
- No pigment production
- No growth on paranitrobenzoic acid L–J media
- Weakly catalase—positive
- Niacin—positive
- Nitrate reduction—positive
- Susceptibility to pyrazinamide
- Resistant to TCH

■ Describe the sensitivity testing methods.

Sensitivity testing can be done by:

1. Resistance ratio method
2. Absolute concentration technique
3. Proportion method
4. BACTEC method
5. MODS assay

Resistance Ratio Method

- L–J slant with doubling concentration of drugs are inoculated with test strain and standard strain. Minimum inhibitory concentration (MIC) is calculated (L–J with lowest concentration of drug showing 0–20 colony)
- Ratio of MIC of test strain to MIC of known strain is calculated. The results are interpreted in the following ways:
 - Ratio of 8 is reported as resistance
 - Ratio of 4 is reported as doubtful resistance
 - Ratio of 1 or 2 is reported as sensitive

Absolute Concentration Technique

In this method MIC of only test strain is calculated.

Proportion Method

Here number of colonies growing on L-J with and without drug is compared. Strain is reported as resistant if more than 1% of the bacteria grow on L-J with drug.

BACTEC Method

Liquid medium containing drugs are used for antibiotic susceptibility testing (AST) with radiometric growth detection.

■ List nucleic acid detection methods followed for identifying *Mycobacterium* spp.

Nucleic acid detection methods are:

- Polymerase chain reaction
- Ligase chain reaction
- Strand displacement amplification
- Nucleic acid sequence base amplification
- DNA probes (Gene and line probes)
- Microarray (DNA chip)

■ Which antibody detection methods are employed for identifying *Mycobacterium* spp?

Ab detection methods are:

- Enzyme-linked immunosorbent assay
- Radioimmunoassay
- Latex agglutination

■ Discuss animal inoculation method for identifying *Mycobacterium* spp.

- Animal inoculation method was used in the past for diagnosis, but is not used now
- Sample is inoculated intramuscularly into the thigh of two guinea pigs
- Animal is weighed before and regularly after inoculation
- Development of disease in animals shows progressive weight loss and positive tuberculin test
- One animal is killed after appearance of disease and autopsied
- Autopsy findings are:
 1. Caseous lesion at inoculation site
 2. Enlarged draining lymph nodes
 3. Tubercles in various organs
 4. Kidneys remain unaffected
 5. Smears from exudates are AFB-positive

Disadvantages of animal inoculation

1. It resembles culture and sensitivity testing methods, but culture technique is safer and simpler
2. Animal maintenance is not cost-effective
3. Excretion of bacilli in urine of animal may be hazardous

■ How is tuberculin test useful for detecting *M. tuberculosis*? Mention the uses of tuberculin test.**Tuberculin Test (also called Mantoux test)****Principle**

It is Type IV hypersensitivity reaction.

Procedure

- Purified protein derivative (PPD) is inoculated intradermally on forearm.
- Site observed after 72 hours for appearance of area of erythema and induration.

Interpretation

- Erythema and induration
 - About 10 mm indicates positive test
 - Below 5 mm indicates negative
 - Between 5 and 9 mm indicates doubtful test
- False positive: Atypical mycobacterial infection
- False negative:
 - Early tuberculosis
 - Miliary tuberculosis
 - Advanced tuberculosis
 - Immunosuppressive individuals
 - Severe malnutrition
 - Measles

Uses

1. To diagnose active infection in infants
2. To select population for BCG vaccination
3. It is used as an indication of successful BCG vaccination
4. To measure prevalence of infection in community

■ **Differentiate between *Mycobacterium tuberculosis* and *Mycobacterium bovis*.**

M. tuberculosis and *M. bovis* can be differentiated based on their distinctive features, enumerated in Table 46.1.

■ **Name the laboratory diagnostic methods used for detecting tuberculosis.**

Laboratory methods used for detecting tuberculosis are presented in Table 46.2.

Table 46.1 Differences between *M. tuberculosis* and *M. bovis*

	<i>M. tuberculosis</i>	<i>M. bovis</i>
Morphology	Slender, straight or slightly curved beaded	Stout, straight
Staining	Barred or beaded appearance	Uniformly stained
Colony	Dry, rough, raised, buff coloured	Moist, smooth, flat, white
Growth on L-J	Eugonic growth	Dysgonic growth
Growth on L-J with glycerol	Improved	Inhibited
Nitrate reduction	Positive	Negative
Niacin test	Positive	Negative
Sensitivity to pyrazinamide	Positive	Negative
Sensitivity to TCH	Negative	Positive
Animal pathogenicity	Pathogenic to guinea pigs	Pathogenic to guinea pigs and rabbits

L-J = Lowenstein-Jensen medium.

Table 46.2 Laboratory diagnosis of tuberculosis

Microscopy	Z-N stain and fluorescent stain, LED fluorescent microscopy
Culture	<ul style="list-style-type: none"> • Solid media—L-J • Liquid—Middle brook media, MGIT, MODS assay • BACTEC
Identification	Biochemical tests
AST	Molecular methods – DNA probes, Microarray <ul style="list-style-type: none"> • Resistant ratio method • Absolute concentration method • Proportion method • BACTEC • MODS assay
Nucleic acid detection	PCR, LCR, NASBA, SDA, DNA probes
Ab detection	ELISA, RIA, latex agglutination
Skin test	Tuberculin test
Animal Inoculation	In guinea pig
Other tests	<ul style="list-style-type: none"> • Adenosine deaminase estimation • Tuberculostearic acid detection • Bromide partition test

LCR = ligase chain reaction, L-J = Lowenstein-Jensen medium, NASBA = nucleic acid sequence base amplification, SDA = strand displacement amplification

■ What kinds of drugs are suggested for treatment of tuberculosis?

Bactericidal and bacteriostatic drugs are available for treating tuberculosis.

- Bactericidal—Rifampicin, isoniazid, pyrazinamide, streptomycin
- Bacteriostatic—Ethambutol, cycloserine, capreomycin, kanamycin, ciprofloxacin

■ What prophylactic measure can be adopted to prevent tuberculosis?

Tuberculosis can be prevented by vaccination; the details of the vaccine administered are:

- BCG: Bacille Calmette-Guérin
- Type: Live attenuated vaccine
- Strain used: Strain of *M. bovis*
- Route: Intradermal - over the deltoid
- Age: As soon as possible after birth
- Schedule: Single dose, booster doses are not necessary as tuberculosis is common in India and natural infections with tubercle bacilli maintain immunity (act as a booster dose)
- Immunity: Lasts for 10–12 years. The type of immunity produced is cell-mediated immunity
- Other benefits
 - Protects individual from complicated forms of tuberculosis
 - Offers some protection against leprosy and leukaemia
- Adverse effects
 - Local—abscess, ulcer, keloid
 - Regional—lymphadenitis
 - Systemic—fever, otitis media, erythema nodosum
- Contraindication: Patients of AIDS, measles and tuberculosis

47

Chapter

Mycobacteria II: Atypical Mycobacteria

■ What are atypical mycobacteria?

- Mycobacteria other than typical mycobacteria causing a disease resembling tuberculosis are called atypical mycobacteria
- They are also called
 - Nontuberculous mycobacteria (NTM)
 - Mycobacteria other than tubercle bacilli (MOTT)
 - Opportunistic mycobacteria
 - Paratubercle bacilli
 - Tuberculoid bacilli
 - Anonymous mycobacteria
 - Unclassified mycobacteria

■ Classify atypical mycobacteria.

Atypical mycobacteria have been classified into following four groups by Runyon:

1. Photochromogens
2. Scotochromogens
3. Nonphotochromogens
4. Rapid growers

Photochromogens

- These mycobacteria produce colonies that produce yellow–orange pigment when culture is exposed to light for 1 hour in the presence of air and reincubated for 1–2 days
- Colonies are nonpigmented in dark, e.g. *M. Kansasii*, *M. simiae*, *M. marinum*.

Scotochromogens

- These mycobacteria produce yellow–orange-pigmented colonies even in dark-colour intensity may increase in presence of light, e.g. *M. scrofulaceum*, *M. szulgai*.

Nonchromogens

- These mycobacteria do not produce pigment even on exposure to light, e.g. *M. avium*, *M. intracellulareae*, *M. xenopi*, *M. ulcerans*.

Rapid Growers

- This is a group of mycobacteria capable of growing rapidly, i.e. within 7 days of incubation
- They include some species of photochromogens, scotochromogens, and nonchromogens, e.g. *M. fortuitum*, *M. chelonae*.

■ What are the differences between atypical and typical mycobacteria?

Atypical mycobacteria differ from typical mycobacteria in a number of features. These are listed in Table 47.1.

Table 47.1 Differences between atypical and typical mycobacteria

<i>Atypical</i>	<i>Typical</i>
Opportunistic pathogens	Obligate pathogens
Some are rapid growers	All are slow growing
Some produce pigment	Do not produce pigment
Niacin—Negative	Niacin—Positive
Aryl sulphatase—Positive	Aryl sulphatase—Negative
Strong catalase—Positive	Weak catalase—Positive
Nonpathogenic to guinea pigs	Pathogenic to guinea pigs
Resistant to antituberculous drugs	Sensitive to these drugs
Some grow at 25°C and some at 45°C	Growth does not occur below 25°C and above 44°C

■ Name the diseases caused by atypical mycobacteria.

The diseases caused by atypical mycobacteria and their causal agents are given in Table 47.2.

Table 47.2 Diseases and the associated atypical mycobacteria

<i>Disease</i>	<i>Usual agents</i>
1. Pulmonary infection like tuberculosis	<i>M. kansasii</i> , <i>M. avium</i> , <i>M. intracellulare</i> , <i>M. simiae</i> Rare pathogens <i>M. szulgai</i> , <i>M. xenopi</i> , <i>M. chelonae</i>
2. Lymphadenopathy Usually cervical	<i>M. szulgai</i> , <i>M. avium</i> — <i>intracellulare</i> <i>M. scrofulaceum</i> Rarely by <i>M. kansasii</i>
3. Cutaneous/subcutaneous lesions	
a. Chronic ulcers	<i>M. marinum</i>
b. Abscesses	<i>M. fortuitum</i> , <i>M. chelonae</i>
c. Swimming pool granuloma	<i>M. marinum</i>
d. Buruli ulcer	<i>M. ulcerans</i>
e. Surgical wound infections	<i>M. chelonae</i> , <i>M. fortuitum</i>
4. Systemic disseminated disease	<i>M. avium</i> , <i>M. intracellulare</i>

■ Describe the tests used for identifying atypical mycobacteria.

Identification tests for atypical mycobacteria are as follows:

1. Pigment Production

- It is tested by inoculating two slants, one is wrapped with aluminium foil and the other left as it is. Slant is incubated in presence of 15 W bulb for 14 days and observed for pigment production
- Unwrapped slant produces pigment—Photochromogens

- Pigment production occurs even in wrapped slant—Scotochromogens
- No pigment in both slants—Nonchromogens

2. Rate of Growth

Rapid growers grow in a week.

3. Growth at 25°C and 45°C

Most mycobacteria grow at 25°C, *M. xenopi* grows at 45°C.

4. Growth on L-J with Paranitrobenzoic Acid

Growth of the mycobacteria is itself an identifying feature.

5. Growth on TCZ (Thiophen 2-Carboxylic Acid Hydrazide) L-J Medium

Most mycobacteria do not grow.

6. Reaction with arylsulphatase

Most of them are positive.

■ Mention the identifying biochemical features of atypical mycobacteria.

Identification of Important Photochromogens

	Tween 80 hydrolysis	Growth on TCZ (L-J)	Nitrate reduction
<i>M. kansasii</i>	Positive	Negative	Positive
<i>M. marinum</i>	Positive	Positive	Negative
<i>M. simiae</i>	Negative	Positive	Negative

TCZ = Thiophen 2-carboxylic acid hydrazide.

Identification of Important Scotochromogens

	Tween 80 hydrolysis	Aryl sulphatase	Nitrate reduction
<i>M. scrofulaceum</i>	Negative	Positive	Negative
<i>M. szulgai</i>	Negative/positive	Strong positive	Positive

Identification of Important Nonchromogen

	Tween 80 hydrolysis	Growth at 25°C	Growth at 44°C
<i>M. xenopi</i>	Negative	Negative	Positive
<i>M. avium</i>	Negative	Positive	Positive/negative

Identification of Important Rapid Growers

	Aryl sulphatase	Nitrate reduction
<i>M. chelonae</i>	Strong positive in 3 days	Negative
<i>M. fortuitum</i>	Positive in 18 days	Positive

■ How can atypical mycobacterial infections be diagnosed in a laboratory?

- Laboratory diagnostic methods for atypical mycobacterial infections are similar to those of tuberculosis

- Steps in diagnosis of atypical mycobacterial infections include:
 1. Microscopy
 2. Culture on L-J incubated at 25°C, 37°C, 45°C. Slants are observed for—pigment production
 3. Identification by different tests such as nitrate reduction, aryl sulphatase, Tween 80 hydrolysis, and growth at different temperatures (e.g. 25°C, 44°C).
 4. Antibiotic susceptibility testing

■ **What treatment is recommended for diseases caused by atypical mycobacteria?**

Atypical mycobacteria may respond to prolonged treatment with INH, rifampicin, and ethambutol. Other drugs used are clofazimine, quinolones and macrolides.

Mycobacteria III: *Mycobacterium Leprae*

■ Name the causal agent of leprosy. Who described the disease first?

- *Mycobacterium leprae*, causes leprosy, a disease, which affects skin and peripheral nerves
- Leprosy was first described by Hansen (1873), hence it is also known Hansen's disease

■ Mention the characteristic morphological features of *Mycobacterium leprae*.

Morphological Features (Fig. 48.1)

- *Mycobacterium leprae* are straight or slightly curved, slender, acid fast bacilli (AFB)
- Size: $1-8 \mu \times 0.3 \mu$
- They are less acid fast than *M. tuberculosis*, need 5% sulphuric acid to express the reaction
- They are arranged singly or in groups intra- and extracellularly
- Intracellular bacilli usually form parallel bundles called **globi**, which gives cigar bundle appearance
- Bundle formation is due to a lipid-like substance called **glia**, which bound them together
- In tissue, bacilli resemble cigarette ends
- Histiocytes containing globi are called **foamy cells** or **lepra cells**
- They are nonmotile and noncapsulated



Fig. 48.1 *Mycobacterium leprae*. (Source: Mosby's Dictionary of Medicine, Nursing & Health Professions, 2010, Elsevier.)

■ What is morphological index? How is it useful in determining a patient's response to treatment?

Morphological Index (MI)

- Live bacilli in smear are stained uniformly
- The percentage of such bacilli in tissue is called morphological index

Use

It is helpful to see patient's response to treatment—continued fall indicates response to treatment while fall succeeded by rise indicates drug resistance.

■ What is bacteriological index? How is it calculated?

Bacteriological Index (BI)

- Live bacilli are stained uniformly while dead bacilli appear beaded or fragmented
- Bacteriological index is the total number of live and dead bacilli in an oil immersion field

Table 48.1 Ridley scale for calculation of bacteriological index

BI	Number of AFB in oil immersion field (OIF)
1+	1–10/100 OIF
2+	1–10/10 OIF
3+	1–10/1 OIF
4+	10–100/OIF
5+	100–1000/OIF
6+	More than 1000, clumps/OIF

AFB = acid fast bacilli.

Calculation of Bacteriological Index

- Ridley scale is used for calculation of bacteriological index of smears (Table 48.1)
- For calculation of BI, minimum of four smears should be examined, which should be from different sites
- Each of them is graded by Ridley scale
- It is calculated by total of number of grades (pluses) in all smears and dividing it by number of smears

■ How is *M. leprae* cultivated?

- It is not possible to cultivate lepra bacilli in artificial culture media
- Animals are used for isolation and culture
- Generation time is 13–15 days
- Animals used are mice and nine-banded armadillo

Uses of animals

1. To isolate bacteria
2. To study bacilli
3. To see viability of bacilli
4. To study susceptibility and resistance to antileprosy drugs
5. To study efficacy of vaccines

■ State the advantages and disadvantages on the use of mice and nine-banded armadillo as systems for cultivation of *M. leprae*.

Mice

- Footpads of mice are used for inoculation
- After intradermal inoculation granuloma develops at the site of inoculation in 1–6 months

Advantages

1. It is a handy animal
2. Cost of maintenance is low
3. It is easily available
4. If CMI is suppressed by antilymphocyte serum or thymectomy, mice develop generalized infection like lepromatous leprosy

Disadvantages

1. Following intradermal inoculation, limited multiplication of bacilli occurs, hence yield is low and not sufficient for research purposes

2. Mice have short lifespan, while leprosy has chronic course, hence, it is not suitable to study pathogenesis of leprosy
3. Disease produced is not like lepromatous leprosy

Nine-banded Armadillo (*Dasypus novemcinctus*)

Advantages

1. Following inoculation, extensive multiplication of bacilli occurs, hence yield is more than that of human leprosy and is sufficient for research purposes
2. It has long lifespan (12–15 years), hence it is suitable to study pathogenesis of leprosy
3. Disease produced is like lepromatous leprosy
4. This animal has low body temperature, hence it is highly susceptible to leprosy bacilli

Disadvantages

1. Availability of the animals is only in Southern USA, so in the other parts of the world their cost is high
2. Some armadillos are naturally infected with mycobacteria resembling *M. leprae*
3. Only 40% armadillos are susceptible to infections

■ Under which conditions *M. leprae* can survive and for how long?

- *M. leprae* can survive in humid climate for 9–15 days
- They survive exposure to sunlight for 2 hours

■ How many wall layers are present in *M. leprae*? State their composition.

M. leprae has following four wall layers:

1. Innermost peptidoglycan
2. Next is lipoarabinomannan B (LAM-B)
3. Mycolic acid layer
4. Outer layer is of mycosides containing phenolic glycolipid (PGL)

■ Name the different types of antigens present on *M. leprae*. Mention the uses of antigens—LAM-B and PGL-1.

- Types of antigens present on *M. leprae* are:
 1. Group-specific antigens
 2. Species-specific antigens
- LAM-B and PGL-1 are immunogenic and are used in serodiagnosis. PGL-1 suppresses CMI and protects against host cell enzymes

■ Which disease is caused by *M. leprae* and how is the infection transmitted?

- *M. leprae* causes leprosy, a disease specially affecting skin, nerves, and nasal mucosa
- Mode of infection is exactly not known. Organism may infect via respiratory tract and skin-to-skin contact

■ Describe clinical types of leprosy.

- According to Ridley and Jopling, clinical types of leprosy are:
 - Tuberculoid (TT)
 - Borderline tuberculoid (BT)
 - Borderline (BB)
 - Borderline lepromatous (BL)
 - Lepromatous (LL).

Table 48.2 Differences between the two polar forms of leprosy

	<i>Tuberculoid</i>	<i>Lepromatous</i>
1. Cell-mediated immunity	Good	Deficient/absent
2. Host resistance	High	Low
3. Lesions		
– Number	Few	Many
– Appearance	Macular, dry, scaly lesions	Nodular, shiny lesions
– Sensations	Anaesthetic patch	Little or no loss of sensations
4. Nerve involvement	Early involvement, asymmetrical, thickened peripheral nerves common	Late involvement, symmetrical, less common
5. Infectivity	Usually noninfective form	Highly infective form
6. Bacteria	Paucibacillary form	Multibacillary form
– Skin smears	Scanty or not seen	Plenty in number
– Nasal smears	Not seen	Plenty in number
7. Lepromin test	Positive	Negative
8. Granuloma formation	Common	Absent
9. Plasma cell infiltration	Poor	Common
10. Lymphocyte infiltration of lesion	Common	Not present
11. Autoantibody and antimycobacterial Ab	Rarely produced	More in number
12. Type II lepra reaction	Negative	Positive
13. Prognosis	Good	Bad

- Two polar forms of leprosy are tuberculoid and lepromatous
- Borderline case has lesions of both polar forms
- When lesions are more like tuberculoid, it is called borderline tuberculoid and when like lepromatous, it is called borderline lepromatous
- Development of form depends on resistance and CMI of a person
- Differences between two polar forms are summarized in Table 48.2

■ Explain lepra reaction.

Lepra Reaction

- Course of leprosy shows acute exacerbations due to immune reactions
- Two such reactions are:
 1. Type I
 2. Type II

Type I

- It is also called lepra reaction or reversal reaction
- It is seen in borderline leprosy cases that develop CMI and shift to tuberculoid form
- Lesions are swollen and erythematous along with pain and tenderness

Type II

- It is also called erythema nodosum leprosum (ENL)
- It is observed in lepromatous or borderline lepromatous patients
- It is usually developed when patient is under treatment

- Crops of tender, inflamed subcutaneous nodules appear, patient also develops fever, arthralgia and lymphadenopathy
- It is a response to antigens released from dead bacilli

These antigens with precipitating antibody present in serum are responsible for ENL.

■ What is lepromin test? Explain the reaction and mention the uses of the test.

Lepromin Test

- It is a skin test for delayed hypersensitivity and used to study immunity in leprosy patients
- Ag used is Mitsuda's Ag or bacillary lepromin

Procedure

- 0.1 ml of Ag injected intradermally on forearm
- Response shows following two reactions:
 1. **Early or Fernandez reaction**—It is an acute inflammatory area of more than 10 mm in diameter appearing in 24–48 hours and disappears in 3–4 days
 2. **Late or Mitsuda's reaction**—It appears 3–4 weeks after injection in the form of nodule, which may ulcerate or subside in few weeks
- Mitsuda's reaction is more meaningful as it is a manifestation of CMI, induced by lepromin while Fernandez reaction indicates past reaction

Uses

1. **To classify lesions of leprosy**
 - It is positive in tuberculoid and negative in lepromatous
2. **To assess prognosis and response to treatment**
 - Positive reaction indicates good prognosis and negative reaction indicates bad prognosis
 - Conversion of negative test to positive indicates improvement
3. **To assess resistance of an individual to leprosy**
 - Those showing positive reaction are capable of responding to *M. leprae* infection
4. **To verify candidate lepra bacillus**
 - Vaccine strain should give matching results to standard lepromin

■ Discuss in brief laboratory diagnosis of leprosy.

Specimens

- **Skin clippings**—taken from peripheral margin of lesion, ear lobules, forehead, cheek, and chin
- **Nasal samples**—include nasal scraping, nasal swab, nasal washing and nasal blow
- **Skin nerve biopsy**
- **Lymph node puncture**

Collection

- **Skin clipping:** Skin is cleaned with spirit swab and pinched to minimize bleeding
 - 5 mm long cut is taken with scalpel
 - After wiping blood, scalpel blade is turned at right angle transversely to scrape sides and bottom of cut to get tissue pulp
- **Nasal scrapings:** A blunt scalpel is used to scrape septum to remove a piece of mucous membrane, which is teased to form smear
- **Nasal swab:** Cotton swab is inserted in nasal cavity, rubbed and rotated against upper part of nasal septum and used for smears
- **Nasal washings:** Few drops of saline are added to nose, after a few minutes patient is asked to blow hard on cellophane paper—mucous pieces from washings are used for preparation of smears

- **Nasal blow:** Patient is asked to blow hard on cellophane paper; mucous is collected to prepare smears

Microscopic Examination

- Smears stained by modified Z-N stain by using 5% sulphuric acid and observed for acid-fast bacilli
- Grading of smear is done and bacteriological and morphological index is calculated

Culture

- Footpads of mouse are used for isolation of bacilli
- Intradermal inoculation develops granuloma in 1–6 months

Serological Tests

Abs are detected against phenolic glycolipid Ag by

- Latex agglutination test
- ELISA

Lepromin Test

Mitsuda's reaction is not used for diagnosis but to see resistance of patients

■ What treatment measures can be adopted for curing leprosy?

- For **paucibacillary leprosy**—rifampicin, 600 mg once a month and dapsone 100 mg daily for 6 months
- For **multibacillary leprosy**—rifampicin, 600 mg once a month dapsone 100mg daily and clofazimine 50 mg daily for 2 years

49

Chapter

Spirochaetes

■ What are spirochaetes?

Spirochaetes are spiral, motile, flexible bacteria, characterized by presence of endoflagella.

■ Present taxonomic classification of spirochaetes.

Classification

Family: Spirochaetaceae and Leptospiraceae



Genera: *Spirochaeta*
Treponema
Borrelia

Leptospira

■ Give the name of medically important spirochaetes.

Medically important spirochaetes are:

1. *Treponema*
2. *Borrelia*
3. *Leptospira*

■ Mention the morphological features and name the species belonging to the genus *Treponema* along with the diseases they cause.

- *Treponema* spp. are slender spirochaetes with fine spirals and pointed or rounded ends
- 'Treponemes'—causing human disease are:

Species	Disease caused
1. <i>T. pallidum</i>	Venereal syphilis
2. <i>T. endemicum</i>	Endemic syphilis
3. <i>T. carateum</i>	Pinta
4. <i>T. pertenue</i>	Yaws

■ Describe the morphological characteristics of *Treponema pallidum*.

Morphological Characteristics (Fig. 49.1)

- It is thin, flexible bacteria twisted spirally around long-axis, with tapering ends
- Size: 6–14 μ long \times 0.2 μ in breadth
- It has 10 regular spirals at regular distance of 1 μ
- It is actively motile with the help of endoflagella

■ Enumerate the methods that can be used for demonstration of *T. pallidum*.

- *T. pallidum* does not take ordinary bacterial stains

- It is best seen by
 1. Dark ground microscope
 2. Silver impregnation method: Fontana—for culture films, Levaditi—for tissue sections
 3. Giemsa—Pale pink
 4. Negative staining using India ink or nigrosin
 5. Electron microscope

■ How can treponemes be grown artificially?

- Pathogenic treponemes do not grow on artificial culture media
- They have to be maintained by serial testicular passage in rabbit, e.g. Nichol's strain
- Nonpathogenic treponemes, e.g. Reiter's strain can be grown in Noguchi's medium

■ Which factors destroy *T. pallidum*?

- *T. pallidum* is a very delicate bacterium
- It is readily inactivated by heating and drying (41–42°C for 1 hour), therefore fomites are of little importance in transmission
- It is killed at 0–4°C in 1–3 days, hence transfusion syphilis can be prevented by storing blood for 4 days in refrigerator before transfusion
- It can be inactivated by common soaps and antiseptics

■ Describe the antigenic structure of *T. pallidum*.

- Antigens of *T. pallidum* are of following two types:
 1. **Nonspecific Ag** - Cardiolipin Ag induces reagin Ab. It is not known whether the Ab is induced by cardiolipin in spirochaetes or is released from damaged host tissue
 2. **Specific Ags**
 - Group specific Ag: Protein Ag—shared by pathogenic and nonpathogenic treponemes
 - Species specific Ag: Polysaccharide in nature

■ Which disease is caused by *T. pallidum*? How is the infection acquired?

- The disease caused by *T. pallidum* is syphilis. It is a sexually transmitted disease
- It enters through minute abrasions on mucosa or skin
- Incubation period is 10–90 days

■ Describe the clinical features of syphilis.

Clinical features can be divided into the following three stages:

1. Primary
2. Secondary
3. Tertiary

Primary

- This stage is characterized by the presence of chancre at the site of entry of spirochaetes; usually it is genital, rarely extragenital seen in mouth and nipples
- The chancre is painless, avascular, indurated circumscribed, superficial ulcer, hence called hard chancre



Fig. 49.1 *Treponema pallidum*.

- Chancre is covered with thick glairy exudate rich in spirochaetes
- Regional lymph nodes are enlarged, rubbery, nontender and discrete
- Chancre heals leaving a thin scar
- The infection is rarely contracted by doctors and nurses nonvenereally, in whom chancre appears on fingers

Secondary

This stage occurs due to widespread multiplication and dissemination of treponemes through blood

- It manifests as mucocutaneous eruptions in the form of roseolar or papular rash and condylomata at mucocutaneous junctions
- Osseus, ophthalmic, meningeal involvement may occur
- After secondary lesion disappears, there is a period called "latent syphilis" during which the diagnosis can be made only by serological tests

Tertiary

This stage manifests as:

- Aneurysms
- Chronic granulomata (gummata)
- Meningovascular involvement
- General paralysis of the insane
- Congenital syphilis—*T. pallidum* can cross-placental barrier after 4 months of gestation
 - Obstetric history of untreated female is typical one of abortion and stillbirth, followed by live births with stigmata of syphilis and finally healthy infant

■ Describe the procedures followed and examinations conducted in the laboratory diagnosis of syphilis.

Specimens

Blood, exudate from lesions, lymph node aspirate

Collection

- Exudates and aspirate are collected for microscopy
- Exudates—with all precautions, the lesion is cleaned with warm saline and margins scraped and pressure applied to base of lesion. Exudate are collected without admixture of blood
- Lymph node aspirate—it can be aspirated with a syringe
- Blood—is collected for serological test by venepuncture in a plain bulb

Transport

Samples for microscopy should reach laboratory immediately after collection.

Microscopic Examination

- **Dark ground microscopy** – Specimen placed directly on slide to observe motile slender, spirals with pointed ends
- **Direct fluorescent antibody technique** – Smear is prepared from specimen and stained with fluorescein-labelled monoclonal Ab. If *T. pallidum* is present, fluorescence is observed. It has more sensitivity than dark ground microscopy
- **Treponemes in tissue** – These can be demonstrated by immunofluorescence or silver impregnation technique such as Levaditi stain

Serological Tests

- **Standard tests (nonspecific)**
- **Treponemal tests (specific)**
 - VDRL, Kahn and Wasserman tests
 - By using Reiter's strain - RPCFT
 - By using Nichol's strain
 - **Live strain** - TPI
 - **Killed treponema** - TPA, TPTA, FTA
 - **Extract of treponemes** - TPHA, TP- EIA

1. Standard Tests

- These are also called reagin Ab tests
- Standard tests include:

VDRL (Venereal Disease Research Laboratory) Test

The test was developed in venereal disease research laboratory in New York

Principle

It is a slide flocculation test, a type of precipitation test

Procedure

Qualitative test

- It is carried out on VDRL tile (tile with multiple concavities)
- Inactivated drop of serum is added to concavity
- A drop of VDRL Ag is then added and mixed with stick
- Rotated manually or mechanically on VDRL rotator fixed at 180 rpm/minute for 4–8 minutes
- Result read under microscope

Quantitative test

- Serial doubling dilutions of serum are prepared and used for the test
- Highest dilution found reactive is taken as titre

Interpretation

- Presence of floccules—Reactive
- Absence of floccules—Nonreactive
- A titre of 1:8 or more is considered as significant

Modifications of VDRL

- Rapid plasma reagin test (RPR)
- Tolidine red unheated serum reagin test (TRUST)
- Automated reagin test (ART)
- Unheated serum reagin test (USR)
- VDRL-ELISA

RPR is the most popular among these, advantages of RPR over VDRL are summarized in Table 49.1.

Biological false positive (BFP) reactions

- Cardiolipin Ag is present in *T. pallidum* and in mammalian tissue so both may induce Ab response. This is a reason for biological false positive VDRL test and it is not a technical error

Table 49.1 Advantages of RPR over VDRL

	VDRL	RPR
Specimen	Serum	Serum/plasma
	Serum needs inactivation	No heating of serum needed
Ag	Must be freshly prepared each day before use	Ready and stable for use
Test reading	Needs microscope	Can be read with naked eyes
Technique	Complicated	Easy and quick

- Positive reaction is obtained in cardiolipin test with negative results in specific treponemal test in the absence of treponemal infection
- Types—acute and chronic
 - Acute BFP reactions last for a few weeks to a month and chronic for more than 6 months
 - Acute BFP—are observed in infections, inflammations, injuries
 - Chronic BFP—are observed in
 - Autoimmune diseases, e.g. SLE
 - Malaria
 - Lepromatous leprosy
 - Infectious mononucleosis
 - Hepatitis
 - Tropical eosinophilia

Kahn Test

It is tube flocculation test, rarely used now.

Wasserman Test

This is complement fixation test, rarely used now.

2. Treponemal Tests

These include the following tests:

- Test using Reiter's strain Ag
- Tests using Nichol's strain as Ag

Test Using Reiter's Strain Ag

- This test is also called **Reiter's protein complement fixation test (RPCFT)**
- It uses Ag from cultivable treponemes. They are not in use now

Tests Using Nichol's Strain as Ag**(a) Test using live strain**

- **Treponema pallidum immobilization (TPI) test**
 - Test uses live treponemes (Nichol's strain)
 - Patient serum is incubated along with complement and treponemes
 - In presence of Abs in sera treponemes are immobilized
 - Most specific, but complex so done in very few laboratories

(b) Tests using killed treponemes

- **Fluorescent treponemal antibody (FTA) test**
 - It is indirect immunofluorescence test using treponemal Ag
 - Serum is allowed to react with the smear prepared with Nichol's strain

- Excess serum is then washed and smear treated with antihuman Ig fluorescent conjugate
- Smear is examined under fluorescent microscope
- Treponemes are seen as fluorescent objects if test is reactive
- Test has good sensitivity 80%, 100%, 95%, in primary, secondary and tertiary syphilis
- Can be modified as fluorescent treponemal antibody absorption (FTA-ABS) test and IgM-FTA
- ***Treponema pallidum* agglutination (TPA) test**
 - Killed suspension of treponemes is mixed with test sera and incubated
 - In presence of Ab, treponemes are found agglutinated under dark ground microscope
 - Not very specific and gives false positive, hence not used now for diagnosis
- ***Treponema pallidum* immune adherence (TPIA) test**
 - Killed suspension of treponemes is mixed with serum, complement, and fresh whole blood and incubated
 - In presence of Ab treponemes adhere to erythrocytes
 - Not used now for diagnosis

(c) Tests using extracts of treponemes

- ***Treponema pallidum* haemagglutination (TPHA) test**
 - Ag is absorbed on surface of RBC
 - When Ag is mixed with sera, in presence of Ab, haemagglutination occurs

Advantages

1. Can be done on CSF
 2. Simple to perform routinely
- **Enzyme immunoassay (EIA) test** – Ultrasonicate of *T. pallidum* Ag coated on wells. Ab is detected by EIA.

■ Which antibiotics are used in the treatment of syphilis?

Syphilis can be treated by the following antibiotics:

- Penicillin
- Doxycycline
- Ceftriaxone

■ Mention nonvenereal treponematoses.

Nonvenereal treponematoses is a group of infections caused by a *Treponema* and is transmitted nonsexually. It includes:

- Endemic syphilis
- Yaws
- Pinta

■ What is endemic syphilis?

- Causal agent is *Treponema endemicum*
- Disease occurs in young children, living in poor hygienic conditions
- It is transmitted from person to person by use of contaminated utensils of drinking and eating
- Primary lesions are rare, may be seen on nipples of mothers infected by their infants. Other clinical features are like secondary and tertiary stages of syphilis
- Laboratory diagnosis is similar to that of syphilis

■ Describe treponemal infections—Yaws and Pinta.

Yaws

- **Aetiological agent:** *Treponema pertenue*
- **Transmission:** Through direct contact with lesions, fly may transmit infection
- **Clinical features:** Primary lesion—extragenital papule breaks to form ulcer
 - Lesions usually occur on legs
 - Secondary and tertiary stages are similar to syphilis except that cardiovascular and neurological symptoms are rare
- **Laboratory diagnosis:** Similar to syphilis

Pinta

- **Aetiological agent:** *Treponema carateum*
- **Transmission:** Through direct person-to-person contact
- **Clinical features:** Primary lesion—extragenital papule develops into lichenoid patch. Skin lesions are seen as hypo- or hyperpigmentation
- **Laboratory diagnosis:** Similar to syphilis

■ Mention the general features of the genus *Borrelia*? Name its species.

- *Borrelia* spp. are large, motile, spirals with irregular, wide and open curves
- Species of *Borrelia* are: *B. recurrentis*, *B. burgdorferi* and *B. vincenti*

■ Enumerate the morphological features of *Borrelia*.

Morphological Features (Fig. 49.2)

- *Borrelia* spp. are 8–20 μ long, 0.2–0.5 μ in width
- They have 15–20 endoflagella
- They have coarse irregular, uneven, wide, open spirals with pointed ends
- Demonstration: They can be seen with light microscope by using Giemsa stain
- Motility: Actively motile

■ Discuss pathogenicity of *Borrelia*. Also write the associated clinical infections.

Pathogenicity

- *B. recurrentis*, *B. duttoni* and few other species cause relapsing fever
- *B. burgdorferi* is a causative agent of Lyme's disease
- *B. vincenti* along with *Fusobacteria* causes Vincent's angina

Relapsing fever (Table 49.2)

1. Louse borne
2. Tick borne

Clinical features

- After incubation period patient gets fever of sudden onset, febrile period lasts for 3–5 days followed by afebrile period of 4–10 days



Fig. 49.2 *Borrelia*.

Table 49.2 Louse-borne and tick-borne relapsing fever

	Louse borne	Tick borne
1. Agent	<i>B. recurrentis</i>	<i>B. duttoni</i>
2. Vector	Body louse	Tick
3. Route of entry	Not by bite but because of being crushed and rubbed into abraded skin	By bite of tick and through their discharge
4. Shedding	Not shed in saliva and discharges	Shed in saliva and discharges
5. Transovarial transmission in vector	Not transmitted	Transmitted
6. Clinical features	Epidemic, more severe	Endemic and less severe

- After that another bout of fever sets in 3–10 days
- Recurrences are common
- *Borreliae* are present in blood during febrile period and are absent during afebrile period.
- Reason for relapse is antigenic variations of *Borrelia*. Recovery occurs after a number of relapses due to development of antibodies against all antigenic variants.

■ How is *Borrelia* infection diagnosed in a laboratory?

Specimen

Blood.

Microscopic Examination

- Wet film of blood drop under dark ground microscope shows motility and typical morphology
- Giemsa stain or Leishman stained blood smear also demonstrate *Borrelia*

Culture

Difficult and unreliable.

Animal Inoculation

- 1–2 ml blood is inoculated intraperitoneally in white mice
- *Borreliae* multiply and appear in blood within 2 days
- Smears prepared from blood are collected from tail vein and examined for *Borrelia* spp.

■ Which antibiotics are suitable for treating *Borrelia* infection?

Antibiotics suitable for treating *Borrelia* infection are: tetracycline, chloramphenicol, penicillin and erythromycin.

■ Describe Lyme's disease.

- Lyme's disease is caused by *B. burgdorferi* and is transmitted by bite of ixodid ticks that becomes infected while feeding on infected animals
- Organism grows in midgut of tick and transmission occurs by regurgitation of gut contents during blood meal
- It occurs in the following three stages:

1. Localized Infection

- Characterized by erythema chronicum migrans
- It is a macule at the site of bite surrounded by redness and induration associated with fever, headache and chills

2. Disseminated Infection

In this, infection spreads to a variety of organs causing neurological and cardiac symptoms such as:

- Meningitis—headache
- Encephalitis—myopericarditis
- Peripheral neuropathy—heart failure

3. Persistent Infection

Characterized by chronic arthritis and arthralgia.

■ How can Lyme's disease be detected in a laboratory?

Specimens

Blood, cerebrospinal fluid (CSF), specimen from local lesion.

Collection

By using usual procedure.

Microscopic Examination

The following methods can be used:

1. Giemsa-stained peripheral blood smear or CSF sediment smears
2. Dark ground microscopy
3. Phase contrast microscopy
4. Silver impregnation
5. Immunofluorescence

Culture

Slow and difficult.

Serology

Ab detection by

- Immunofluorescence test and ELISA
- Abs take 1–2 months to appear

Nucleic Acid Detection

PCR for detection of nucleic acid in tissue and body fluids.

■ Which antibiotics can be used to treat Lyme's disease?

Antibiotics that can be used to treat Lyme's disease are: doxycycline, amoxycillin and cefuroxime.

■ What is Vincent's angina? How is it diagnosed and what treatment is offered?

Vincent's Angina

- *B. vincenti* is commensal of oropharynx
- It causes Vincent's angina (ulcerative oropharyngitis) and gingivostomatitis along with anaerobic fusiform bacteria

Laboratory Diagnosis

Demonstration of causal agent in exudate from clinical lesions.

Treatment

Penicillin and metronidazole.

■ **Mention the morphological characteristics of *Leptospira*.**

Morphological Characteristics (Fig. 49.3)

- *Leptospira* are slender, spirals about $6-20\ \mu \times 0.1\ \mu$ in size
- They have closely set spirals and hooked ends
- They are actively motile, show spinning and bending or sharply flexing movements



Fig. 49.3 *Leptospira* spp.

■ **List the species of *Leptospira*.**

- Species of *Leptospira* are *L. interrogans* and *L. biflexa*
- *L. interrogans* is a potential pathogen of humans and *L. biflexa* is saprophyte

■ **Name the serogroups of *L. interrogans*.**

- *L. interrogans* has 26 serogroups each with different serovars
- Some serogroups are:
 - *Icterohaemorrhagiae*
 - *Canicola*
 - *Australis*
 - *Pyrogenes*
 - *Hebdomadis*

■ **Mention the cultural characteristics of *Leptospira*.**

Cultural Characteristics

- Difficult to culture, not done routinely
- Aerobic and microaerophilic
- In semisolid media they grow few millimeters below the surface
- Optimum temperature $25^{\circ}-30^{\circ}\text{C}$, pH 7.2

Media

- Korthof's media
- Stuart's media

- Fletcher's media
- Ellinghausen, McCullough, Johnson, Harris (EMJH)
- Chick embryo—grow in blood of allantoic vessels

In absence of cultures, the organism can be obtained through **animal inoculation**. The inoculation is done intraperitoneally in guinea pigs.

- **Describe pathogenicity of *Leptospira*, including the clinical features of the disease.**

Pathogenicity

- *Leptospira* causes zoonotic disease—**leptospirosis**
- Transmission: Transmitted to man by direct and indirect contact with water contaminated by urine and faeces of carrier animal

Entry

- Through cuts and abrasions on skin
- Through intact mucus membrane of mouth, nose and eyes

Clinical Features

- It may be asymptomatic, detected by serological tests
- It can cause nonicteric or icteric leptospirosis
- Nonicteric—presents as fever, myalgia, headache, nausea, vomiting, and splenomegaly and aseptic meningitis
- Icteric—presents as hepatic involvement causing jaundice
- Vascular involvement or renal involvement can occur causing disseminated intravascular coagulation (DIC) or acute renal failure

- **How is *Leptospira* detected in a laboratory?**

Leptospira is detected in a laboratory by the following examinations/tests:

Specimens

Blood, urine, CSF.

Collection

As *Leptospira* spp. die in acidic urine, urine should be made alkaline or it should be examined immediately.

Processing of Specimen

Microscopic Examination

Fluorescent Ab technique, silver impregnation technique, dark ground microscopy.

Culture

- Difficult to culture, therefore it is not done routinely
- Culture identification is done by agglutination with type specific sera. Generally, it is done by reference laboratory

Serology

- It is the usual method of laboratory diagnosis
- Abs appears at the end of a week

- They can be detected by the following two types of tests:
 1. Screening tests or genus specific tests
 2. Specific tests or confirmed tests

Genus specific tests

They use Ag from nonpathogenic strains, tests are:

- CFT
- Agglutination
- Indirect immunofluorescence
- ELISA
- Rapid dip stick

Type specific test

Macroscopic and microscopic agglutination tests

- **Macroscopic:** Formalized suspension of prevalent *Leptospira* serovar is used for macroscopic agglutination of test sera
- **Microscopic:** Live culture of different serotypes are used and agglutination observed under low power of dark field

■ Which antibiotics can be used in the treatment of *Leptospira* infections?

Leptospira spp. are sensitive to penicillins and tetracyclines.

50

Chapter

Actinomyces and Nocardia

■ What are the common features of *Actinomyces* and *Nocardia*?

Actinomyces and *Nocardia* are filamentous Gram-positive bacteria resembling fungi.

■ Name the species belonging to the genus *Actinomyces*.

Species belonging to the genus *Actinomyces* are as follows:

- *A. israelii*
- *A. naeslundii*
- *A. viscosus*
- *A. odontolyticum*
- *A. meyeri*

■ Mention the characteristic morphological features of *Actinomyces*.

Morphological Characteristics (Fig. 50.1)

- Thin, Gram-positive branching filaments, straight or slightly curved with varying length
- Also shows beaded appearance

■ Mention the cultural characteristics of *Actinomyces*.

Cultural Characteristics

- Thioglycollate broth and brain-heart infusion agar are used and incubated anaerobically at 37°C
- *A. israelii* forms fluffy ball at the bottom of thioglycollate broth
- Brain-heart infusion agar shows spidery colony after 48–72 hours; become heaped-up white, irregular and smooth colonies, giving molar teeth appearance

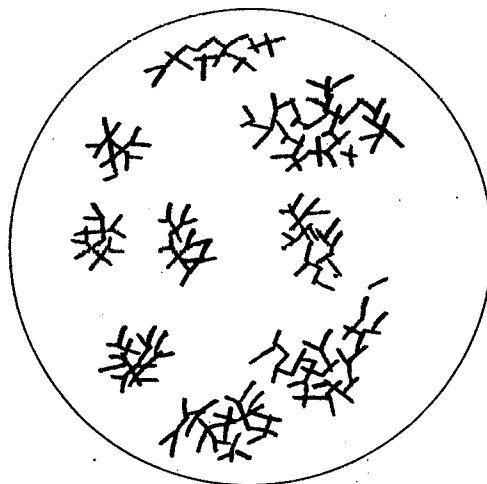


Fig. 50.1 Actinomycetes.

■ Which diseases are caused by *Actinomyces*?

Actinomyces cause the following diseases:

- Actinomycosis
- Gingivitis and periodontitis
- Mycetoma

■ Describe the clinical features of actinomycosis.

- Actinomycosis is a chronic granulomatous disease characterized by development of indurated swelling in connective tissue, suppuration and discharge of sulphur granules through multiple sinuses
- It occurs in four forms, all of which cause abscesses (collections of pus)

Clinical Forms

- **Cervicofacial:** Indurated lesions are seen on cheek and submaxillary region
- **Thoracic:** Lesion is present in lungs that may involve pleura, pericardium and spread outwards to chest wall
- **Abdominal:** Lesion is around caecum with involvement of neighbouring tissue and the abdominal wall. Infection also spreads to liver via portal vein
- **Pelvic:** It is common in females using intrauterine devices

■ What laboratory diagnostic procedures and screening methods should be adopted to identify *Actinomyces*?

Specimens

Pus, sinus discharge, granules, infected tissue, sputum.

Collection

- Pus is collected in a test tube containing normal saline, granules sediment at bottom after shaking, which can be used for further examination
- Granules can be collected by applying gauge pads to sinuses
- They can also be collected by using probe or loop

Gross Examination of Granules

Granules are white to yellow in colour and 0.5–5 mm in size.

Microscopic Examination

- Granules are crushed between two slides and used for Gram stain
- Stained smears show network of thin Gram-positive filaments surrounded by peripheral zone of swollen radiating club-shaped structures representing **sunray appearance**

Culture

- Washed granules are inoculated in thioglycollate broth and brain–heart infusion agar and incubated anaerobically at 37°C
- *A. israelii* forms fluffy ball at bottom of thioglycollate broth
- Brain–heart infusion agar shows spidery colony after 48–72 hours; become heaped-up white, irregular, smooth, molar teeth-like appearance. Identification can be done by fluorescent Ab technique

■ How can *Actinomyces* infection be treated?

Actinomyces infection can be treated by surgical removal of affected tissue with antibiotics such as penicillin, tetracycline, erythromycin, chloramphenicol.

■ Mention the names of species of the genus *Nocardia*.

Species of the genus *Nocardia* are: *N. asteroides*, *N. brasiliensis* and *N. caviae*

■ **Mention the morphological features that characterize *Nocardia*.**

Morphological Features

- These are Gram-positive filamentous bacteria; also show coccoid forms
- They are acid fast when decolourized with 1% sulphuric acid

■ **Which cultural characteristics are typical of *Nocardia*?**

Cultural Characteristics

- Strict aerobe
- Grow on nutrient agar, brain–heart infusion agar and Sabouraud's dextrose agar
- Colonies—dry, granular, wrinkled and may be pigmented

■ **Describe pathogenicity of *Nocardia* infection.**

- The disease caused by *Nocardia* spp. is nocardiosis
- It occurs in the following two forms:

1. Pulmonary Form

- It usually originates as primary pulmonary infection
- It may also show miliary pattern, lobar pneumonia, abscess, and pleural effusion
- It may spread to other organs and occurs as opportunistic disease in immunocompromised individuals including AIDS

2. Cutaneous Form

Mycetoma is characterized by formation of swelling, sinus formation and discharge of granules.

■ **How is laboratory diagnosis of *Nocardia* infection performed?**

Specimens

Pus, sputum and granules.

Collection

Sample should be collected in a sterile container and transported to laboratory as early as possible.

Microscopic Examination

- Gram stain—Gram-positive filamentous bacteria
- Z–N stain with 1% sulphuric acid—acid fast bacteria

Culture

Specimen is inoculated on nutrient agar, brain–heart agar and Sabouraud's dextrose agar. Dry, granular, wrinkled (may be pigmented) colonies are further identified by using biochemical tests such as casein hydrolysis, tyrosine hydrolysis and xanthine hydrolysis.

■ **What treatment is suggested for checking *Nocardia* infections?**

Nocardia infections can be treated through drainage, surgery and antibiotics like amikacin, minocycline and cefotaxime.

■ **Mention the distinguishing features of *Actinomadura* and *Streptomyces*.**

Actinomadura

- Species: *A. madurae*, *A. pelletierii*
- They cause mycetoma

- Morphologically, they are fine, intertwining, branched, nonfragmented filaments resembling culturally with *Nocardia*, can be differentiated further by biochemical reactions

Streptomyces

- Species: *S. somaliensis*
- It causes mycetoma
- It is Gram-positive filamentous bacteria, which may fragment

Rickettsiaceae and Bartonellaceae

■ Mention the general features of the family Rickettsiaceae.

General Features

- The family Rickettsiaceae is named so in honour of Howard Taylor Ricketts, the scientist who worked on spotted fever and died of typhus fever, contracted while working on the organism
- These are small Gram-negative bacilli, obligate intracellular parasites and are transmitted to humans by arthropods (except Q fever)
- Infections (except Q fever, ehrlichiosis) manifest as fever, rash and vasculitis
- They are grouped depending on clinical features, epidemiology and some other characters such as cultural characters, vectors, etc.

■ Which genera belong to the family Rickettsiaceae? Name the species belonging to each genera.

The genera of the Rickettsiaceae and species of each genera are:

Family: Rickettsiaceae

Genera: *Rickettsia*

Orientia

Coxiella

Ehrlichia

Species: *Rickettsia*: *R. prowazekii*

R. typhi

R. rickettsii

R. conori

R. australis

R. siberica

R. akari

***Orientia*:** *O. tsutsugamushi*

***Coxiella*:** *C. burnetii*

***Ehrlichia*:** *E. sennetsu*

E. chaffensis

E. phagocytophila

■ Mention the morphological features of *Rickettsia* and *Orientia*.

■ Morphological Features

- These are small, Gram-negative, pleomorphic coccobacilli
- Size: about 0.3 μ to 1–2 μ
- Nonmotile, noncapsulated

- Do not stain well with Gram's stain but visible under light microscope when stained with Giemsa, Gimenez, Castaneda and Macchiavello
- Castaneda and Giemsa stain them purple while Gimenez and Macchiavello red
- Cell wall resembles Gram-negative cell wall
- They possess both DNA and RNA
- They divide by binary fission

■ **How are *Rickettsia* and *Orientia* cultured?**

- *Rickettsia* and *Orientia* do not grow in cell-free cultures
- They grow in cytoplasm (typhus group) and in cytoplasmic vacuoles (*Coxiella*)
- The following methods are used for cultivation:
 - Yolk sac of 5–6 days old chick embryo is used—they multiply in cells of membrane surrounding yolk sac
 - Cell cultures—grow in many continuous cell lines as HeLa, HEP-2, Detroit 6, mouse fibroblast but are not satisfactory for primary isolation. *Rickettsiae* grow well in cells that are nonmetabolising
 - Animal inoculation—Guinea pigs and mice are used

■ **Mention the factors affecting survival of *Rickettsia* and *Orientia*.**

- Both *Rickettsia* and *Orientia*, cannot survive longer outside the vector or host
- They are quickly destroyed by heat, drying and disinfectants such as hypochlorites, ethanol, 1% lysol and 2% formalin
- Dried faeces of infected lice may contain infectious forms for months at room temperature
- *Coxiella* may survive pasteurisation and in dried milk for months

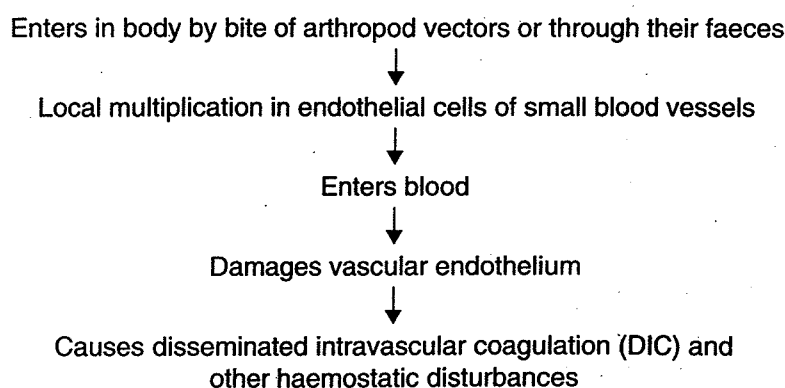
■ **Which antigens are present in *Rickettsia* and *Orientia*?**

Antigens present in *Rickettsia* and *Orientia* are:

- Group-specific soluble Ag—present on surface of all organisms
- Species-specific Ag
- An alkali-stable polysaccharide found in some *Rickettsiae* and in some strains of *Proteus* (OX19, OX2 and OXK), which forms the basis of Weil–Felix reaction, which is used for diagnosis of rickettsial infections

■ **Present an outline sketch showing disease development in infections caused by *Rickettsia* and *Orientia*.**

The way human beings develop *Rickettsia* and *Orientia* infection is presented in Flowchart 51.1.



Flowchart 51.1 Disease development in infections by *Rickettsia* and *Orientia*.

■ **Mention the name of the diseases caused by *Rickettsia* and *Orientia*.**

Diseases caused by *Rickettsia* and *Orientia* are:

1. Typhus fevers
2. Spotted fevers
3. Scrub typhus

■ **Describe various features of rickettsial diseases.**

Rickettsial diseases are described in Table 51.1.

■ **Describe the various laboratory methods of diagnosing *Rickettsia* and *Orientia* infections.**

Specimens

Blood and biopsy from rash.

Methods of Detection

Direct Detection of Rickettsia or Detection of Ag

This can be done by:

- Smear prepared from biopsy of rash stained with Giemsa and Gimenez stain
- Direct immunofluorescence test

Nucleic Acid Detection

PCR.

Isolation

- It should be attempted only in laboratory with safety provisions
- This can be achieved through:
 1. **Animal inoculation:** Blood clot ground in skimmed milk or brain heart infusion broth is inoculated intraperitoneally in male guinea pig or mice, animal is observed for 3 weeks; response (Neill-Mooser reaction or tunica reaction—animal develops fever, scrotal inflammation and testes cannot be pushed back into abdomen because of adhesions between layers of tunica vaginalis) of animal varies in different infections:
 - In rocky mountain spotted fever (RMSF) caused by *R. rickettsii*—fever, scrotal necrosis and animal may die
 - In spotted fevers caused by *R. typhi*, *R. conori*, *R. akari*—fever, tunica reaction
 - In epidemic typhus caused by *R. prowazekii*—fever, no testicular inflammation
 - In scrub typhus caused by *O. tsutsugamushi*—a mouse is preferred, becomes ill, develops ascites and smears from tunica, spleen show *rickettsia*
 2. **Yolk sac:** This can also be used for growing *Rickettsia* and *Orientia*.

Serological methods

1. Weil-Felix test
2. Specific tests using rickettsial Ag

Weil-Felix Test

Principle

- It is a heterophile agglutination test that detects Ab in the patients of rickettsial fever that cross reacts with some strains of *Proteus*, due to sharing of alkali stable carbohydrate Ag of *Rickettsia* and O Ags of nonmotile strains of *Proteus*

Table 51.1 Features of rickettsial diseases

Disease	Geographic distribution	Causative agent	Vector species	Reservoir	Mode of transmission	Clinical features
Typhus fever group						
Epidemic typhus	Worldwide	<i>R. prowazekii</i>	Head louse	Human	<ul style="list-style-type: none"> Louse faeces rubbed through scratches into skin Inhalation of aerosols of dried louse faeces 	Fever, chills, myalgia, headache, rash on trunk, spare palm, face and soles, cloudy state of consciousness
Recrudescence typhus	-	-	-	-	-	Latency and reactivation of epidemic typhus
Endemic typhus	Worldwide	<i>R. typhi</i>	Flea	Rodents	Rat flea faeces rubbed through scratches into skin	Similar but mild illness like epidemic typhus
Scrub typhus						
Scrub typhus	Asia, Australia	<i>O. tsutsugamushi</i>	Mite	Rodents	Mite bite	Fever, headache, rash as in epidemic typhus, eschar lymphadenopathy, atypical lymphocytes, hypovolaemia, hypotensive shock
Spotted fever group						
Rocky mountain spotted fever (RMSF)	USA, South America	<i>R. rickettsii</i>	Tick	Rodents, dog	Tick bite	Fever, headache, macular rash, may be papular, petechial hypovolaemia, hypotensive shock
Rickettsial pox	USA, Russia, South Africa	<i>R. akari</i>	Mite	Mice	Mite bite	Fever and rash like chickenpox
Australian tick typhus	Australia	<i>R. australis</i>	Tick	Rodents	Tick bite	Milder, rash as in RMSF, eschar
South African tick fever	South Africa	<i>R. conorii</i>	Tick	Rodents	Tick bite	Milder, rash as in RMSF, eschar
Indian tick typhus	India	<i>R. conorii</i>	Tick	Rodents	Tick bite	Mild illness as in RMSF eschar
Kenyan tick typhus	Kenya	<i>R. conorii</i>	Tick	Rodents	Tick bite	Mild illness as in RMSF eschar
Boutonneuse fever	Mediterranean	<i>R. conorii</i>	Tick	Rodents	Tick bite	Mild illness as in RMSF eschar
Siberian tick typhus	Siberia	<i>R. sibirica</i>	Tick	Rodents	Tick bite	Mild illness as in RMSF eschar

RMSF = rocky mountain spotted fever.

- *P. vulgaris* OX19, OX2 and *P. mirabilis* OXK
- It is nonspecific and insensitive

Procedure

Sera of patient is mixed with OX19, OX2, OXK Ag in a tube agglutination test and observed for agglutination.

Interpretation

The test results are interpreted as follows:

Disease	Agglutination with		
	OX19	OX 2	OXK
Epidemic typhus	+++	+	-
Brill-Zinsser Disease	Weak, positive or negative with all 03 Ags		
Endemic typhus	+++	±	-
Tick-borne spotted fever	++	++	-
Scrub typhus	-	-	+++

+ = weak positive, ++ = moderate positive, +++ = strong positive, ± = positive or negative, OX19 and OX2 = antigens of species of *Proteus vulgaris*, OXK = antigens of species of *Proteus mirabilis*.

Specific Tests

These include the following tests:

1. Complement fixation test—specific but insensitive
2. Indirect fluorescent Ab test
3. Latex agglutination—for RMSF Positive in acute infection so diagnostic
4. Enzyme immunoassay

■ How can *Rickettsia* and *Orientia* infections be treated?

- Sulphonamide—enhances severity of disease. Sensitive to tetracycline, ciprofloxacin

■ Write a short note on *Coxiella burnetii*.

- *Coxiella burnetii* is a causative agent of Q fever
- It is worldwide in distribution

Characters

- They are smaller than other *Rickettsiae* and are filterable
- In dried faeces or wool they survive for a year at 4°C and in meat for 1 month
- They may survive pasteurisation of milk by holding method
- Yolk sac of embryonated hen's egg is used for culture

Routes of Entry

- Through skin while handling contaminated animal products like meat, hide, wool
- Inhalation of aerosols containing *C. burnetii*
- Drinking infected milk

Reservoirs

Sheep and cattle.

Clinical Features

- It is a zoonotic disease
- It is transmitted among animals by tick, and animals shed them in milk. They are abundant in products of conception which contaminate the environment
- It causes influenza-like illness lasting for 4–7 days
- It spreads to organs causing hepatitis, endocarditis, meningoencephalitis

Laboratory Diagnosis

- Usually by serology
- Isolation not recommended due to hazards of laboratory infection
- Nucleic acid detection—PCR
- Ab detection—indirect immunofluorescence and CFT

Treatment

Doxycycline.

■ Write a short note on *Ehrlichia*.

- *Ehrlichia* are Gram-negative, obligate, intracellular bacilli
- Have affinity towards blood cells — neutrophils, lymphocytes and monocytes and show morula form in phagosome of infected cells
- Transmitted by ticks
- Species: Three species are known, viz.

1. *E. chaffeensis*

- It causes monocytic ehrlichiosis
- It has special tropism to monocytes in tissue and blood
- Clinically shows leucopenia and thrombocytopenia, other features are similar to RMSF

2. *E. sennetsu*

- It causes sennetsu ehrlichiosis (sennetsu meaning glandular fever)
- It shows lymphoid hyperplasia and atypical lymphocytosis.

3. *E. phagocytophila*

- It causes granulocytic ehrlichiosis
- Gram-stained blood films show morula forms of *Ehrlichia*.

Laboratory Diagnosis

- Indirect immunofluorescence test
- Inclusion in monocytes/granulocytes
- PCR

Treatment

Doxycycline.

■ What are *Bartonellae*? Name the human pathogens and mention the salient features of each.

Bartonellae are tiny Gram-negative bacilli usually transmitted by arthropods and invade endothelial cells and blood cells

- Human pathogens are:

1. *B. bacilliformis*

- causes Oroya fever transmitted by sandfly
 - Clinical features: characterized by fever, hepatosplenomegaly, haemorrhages in lymph node and progressive anaemia
 - Laboratory diagnosis: Demonstration can be done by blood smear stained by Giemsa stain

2. *B. quintana*

- causes trench fever or 5 days fever
 - It is exclusively a human disease, without animal reservoir
 - It is transmitted by body louse when infected faeces are rubbed into the skin
 - Clinical features: Fever, headache, malaise, rash on chest, abdomen or back, infection is mild, has slow course and prolonged convalescence
 - Culture: It can be grown on blood agar and so differs from *Rickettsiae*
 - Laboratory diagnosis: PCR

3. *B. henselae*

- causes cat scratch disease
 - Results from contact, scratch or bite of infected cat
 - Clinical features: Cervical and axillary lymphadenopathy, vascular nodule on skin and mucosa
 - Disease severe in HIV and immunocompromised individuals
 - Culture: Isolation can be done by using chocolate agar and Columbia agar
 - Laboratory diagnosis: Lymph node biopsy shows bacilli on Warthin–Starry silver impregnation
 - Disease is self-limited, does not usually need therapy

52

Chapter

Chlamydiae

■ What are *Chlamydiae*?

- *Chlamydiae* are small, obligate, intracellular Gram-negative bacteria
- They are called *Chlamydiae* because of the inclusion produced by them, which encloses nuclei of the cell as a mantle
- They are also called Psittacosis, Lymphogranuloma venereum, Trachoma agents (PLT) or Trachoma inclusion conjunctivitis (TRIC) agents or Bedsoniae. Recently, they are renamed as *Chlamydophila*

■ Mention the general properties of *Chlamydiae*.

General Properties

- *Chlamydiae* are obligate intracellular parasites
- They possess both DNA and RNA
- Their cell wall is similar to that of Gram-negative bacteria but they do not have peptidoglycan
- They lack the ability to synthesize their own ATP and therefore use host's ATP and are called **energy parasites**
- They replicate by binary fission

■ Mention the identifying morphological features of *Chlamydiae*.

Morphological Characteristics

Chlamydiae show two different morphological forms—elementary body (EB) and reticulate body (RB) (Table 52.1).

- They are Gram negative but stain poorly with Gram's stain
- They are nonmotile
- Castaneda stains them blue and Machiavello stains them red
- Giemsa is used for staining inclusions in cell culture
- Inclusions are basophilic

Table 52.1 Differences between elementary body and reticulate body

Elementary body	Reticulate body
1. Extracellular	Intracellular
2. Infectious particle	Metabolically active form
3. Size: 250–350 nm in diameter	Size: 800–1200 nm in diameter
4. Rigidity of cell wall is due to disulphide cross linking	Cell wall lacks disulphide cross linking

Table 52.2 Differences between *Chlamydia trachomatis* and *Chlamydia psittaci*

<i>C. trachomatis</i>	<i>C. psittaci</i>
1. Inclusions possess glycogen, therefore iodine stains it brown	Inclusions of <i>C. psittaci</i> do not have glycogen so do not stain with iodine
2. Compact inclusion	Diffuse, vacuolated inclusion
3. Sensitive to sulphonamides	Resistant to sulphonamides
4. Causes eye and genital infections	Commonly cause pneumonia

■ Differentiate between *C. trachomatis* and *C. psittaci*.

- Distinguishing features of *C. trachomatis* and *C. psittaci* are presented in Table 52.2.

■ Describe the developmental cycle of *Chlamydiae*.

Developmental Cycle (Fig. 52.1)

- Attachment of elementary body to susceptible cell
- Entry into cell by phagocytosis (*Chlamydiae*-dependent modification of endocytic membrane prevents lysosomal fusion and thus escapes degradation)
- EB reorganized into RB, by increasing in size, losing DNA core and breaking disulphide bonds
- Host cell growth gets arrested
- RB divides by binary fission to form pleomorphic bacteria, genus-specific chlamydial Ag becomes associated with cell and form EB in cells
- Developing chlamydial microcolony in the vesicle is called **inclusion**, which is perinuclear and may contain 100–500 elementary bodies
- Nucleus pushed to periphery. Death and lysis of host cell leads to release of EB.

■ To which factors are *Chlamydiae* sensitive?

Chlamydiae are sensitive to routinely used disinfectants and heat.

■ Describe the antigens present in *Chlamydiae*.

Chlamydiae have the following three Ags:

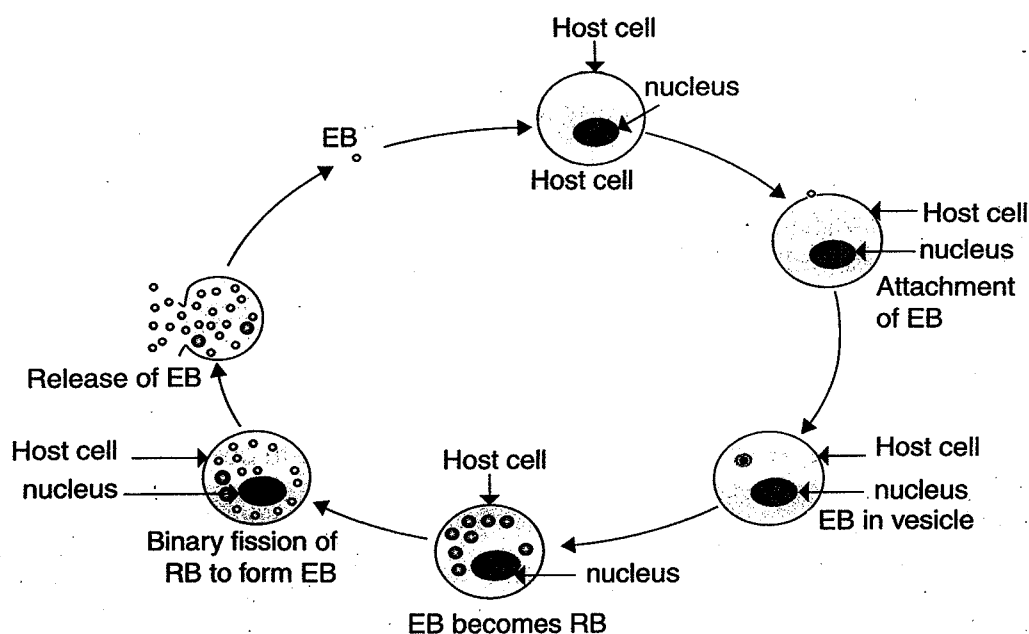


Fig. 52.1 Developmental cycle of *Chlamydiae*; EB = elementary body, RB = reticulate body.

1. Genus-specific Ag

Present in *C. trachomatis* and *C. psittaci*. It is lipopolysaccharide—resembling LPS of Gram – negative bacteria, heat stable, complement fixing, present in EB and RB.

2. Species-specific Ag

Major outer membrane protein Ag.

3. Serotype-specific Ag

- *C. trachomatis* has different serovars
- *C. trachomatis* is classified into two biovars (biological variants)

Biovars

1. TRIC (trachoma and inclusion conjunctivitis)—agent of trachoma and inclusion conjunctivitis
2. LGV (lymphogranuloma venereum)—agent of LGV

These biovars are further classified into serovars.

Serovars

Fifteen serotypes are established by neutralization and immunofluorescence. These are:

1. TRIC—12 serovars—A, B, Ba, C, D to K
2. LGV—3 serovars (L1, L2, L3)

■ How are *Chlamydiae* grown for the purpose of identification?

Chlamydiae can be grown by the following methods:

Animal Inoculation

Mice are preferred. Elementary bodies are observed in smears prepared from lung, peritoneal exudate, spleen and brain after death of the animal

Embryonated Hen's Egg

Yolk sac is used. *Chlamydiae* multiply in endothelial cells and are detected by impression smears stained with Giemsa stain

Cell Culture

- Cells irradiated with metabolic inhibitor are used
- McCoy, HeLa, monkey kidney or mouse fibroblasts cell cultures are used for culture
- Presence of *Chlamydiae* is detected by staining of inclusion

■ Describe the pathogenicity of *Chlamydiae*.

In humans, *Chlamydiae* produce infections of the eye, genital tract and respiratory tract. Table 52.3 briefly states the various infections produced by *Chlamydia* spp.

Eye Infections

Trachoma

It is a chronic keratoconjunctivitis characterized by follicle, papillary, hyperplasia and pannus formation and later on cicatrization. It is one of the causes of blindness. Infection is transmitted from eye to eye by fingers or fomites, flies can play a role in transmission.

Table 52.3 Infections produced by *Chlamydia* spp.

Infection	Disease	Organism	Biovar	Serovar
1. Eye	• Trachoma	<i>C. trachomatis</i>	TRIC	A, B, Ba, C
	• Inclusion conjunctivitis	<i>C. trachomatis</i>	TRIC	D to K
	• Ophthalmia neonatorum	<i>C. trachomatis</i>	TRIC	D to K
2. Genital tract				
Males	Urethritis, epididymitis and proctitis	<i>C. trachomatis</i>	TRIC	D to K
Females	Urethritis, cervicitis, proctitis, salpingitis, infertility, Abortion, stillbirth	<i>C. trachomatis</i>	TRIC	D to K
Male-females	LGV	<i>C. psittaci</i>	—	—
		<i>C. trachomatis</i>	LGV	L1 to L3
3. Respiratory tract	• Pneumonia of infant	<i>C. trachomatis</i>	TRIC	D to K
	• Pharyngitis-pneumonia	<i>C. pneumoniae</i>	—	—
	• Psittacosis	<i>C. psittaci</i>	—	—

— = no biovar and serovar.

Inclusion Conjunctivitis

Characterized by intense hyperaemia, mucopurulent discharge and follicular hyperplasia.

Ophthalmia Neonatorum

It is neonatal form of inclusion conjunctivitis. Infants acquire infection from birth canal of mother and develop conjunctivitis characterized by swelling of eyelid, hyperaemia and purulent infiltration of conjunctiva.

Genital Tract Infections

- In males—nongonococcal urethritis, epididymitis and proctitis
- In females—urethritis, cervicitis, vaginitis, endometritis, salpingitis and infertility. *C. psittaci* causes abortions and stillbirths

Lymphogranuloma Venereum

Characterized by development of painless papule or ulcer on penis in males and fourchette in females. Regional lymph nodes are also enlarged (bubo), become tender and may break open with the formation of sinuses.

Respiratory Tract Infections

Causes pneumonia

- Psittacosis—disease of birds transmitted to man. The organism is excreted in discharges of bird. Humans acquire infection by inhalation and develop influenza-like illness or pneumonia, septicaemia or meningoencephalitis

■ Discuss the laboratory diagnosis of chlamydial infections.

Specimens

Scrapings from mucosa of involved sites such as eyes, urethra, vagina, cervix, blood, sputum, respiratory secretions.

Collection

Scrapings—collected by rubbing cotton swab on mucous membrane to get adequate sample.

Transport

Specimen is transported in sucrose phosphate saline medium with gentamicin and vancomycin. Amphotericin B may also be used for transport of specimens.

Microscopic Examination

Light microscopy to demonstrate inclusions:

- Infection of conjunctiva, urethra and cervix can be demonstrated by showing inclusions when sample is stained with Giemsa or Castaneda stain
- In conjunctival scraping—Halberstaedter–Prowazek bodies (HP bodies) are observed
- In Bubo aspirate—Miyagawa's granulocorpuscles are seen

Antigen Detection

By staining smears with FITC (fluorescein isothiocyanate)-labelled Ab against species-specific Ag. ELISA can also be used to detect Ag.

Culture

Sample is inoculated in mice, cell culture or yolk sac for isolation.

Nucleic Acid Detection

- DNA probes
- PCR—amplifies nucleic acid of bacteria by amplification of DNA
- Chemiluminescence assay
 - It uses acridium—ester—labelled single-stranded DNA probe, which is complementary to RNA of *C. trachomatis*
 - Labelled DNA–RNA hybrid is detected by luminometer

Ab Detection

- CFT—useful in LGV and psittacosis to detect Abs
- Micro-immunofluorescence
- ELISA

Skin Test

- It is used in LGV
- Heat—inactivated LGV grown in yolk sac of egg, injected intradermally on forearm with control
- Positive reaction—inflammatory nodule appears on test arm in two days and reaches maximum 7 mm in four or five days

■ Which antibiotics can be used for treating chlamydial infections?

Antibiotics that can be used are tetracycline, erythromycin and sulphonamides.

53

Chapter

Mycoplasma

■ What are *Mycoplasma*?

Mycoplasma are a group of bacteria that are devoid of cell wall and hence are highly pleomorphic without fixed shape and size. They require sterol for their growth.

Species

- The genus consists of 16 species found in humans
- Established pathogens are—*M. pneumonia*, *Ureaplasma urealyticum* and *M. hominis*

■ Describe the morphological characteristics of *Mycoplasma*.

Morphological Characteristics

- Mycoplasmas are the smallest microorganisms, measuring about 0.2–0.3 μ in diameter
- They are highly pleomorphic because they lack rigid cell wall and so occur in varying shapes such as coccoid, filamentous and bizarre forms (granules, balloon ring, star, disk)
- They show filamentous forms of varying lengths with true branching, which is why they called *Mycoplasma* (*myco* = fungus like and *plasma* = plasticity)
- They do not possess spores and flagella or fimbriae. Some species show gliding motility
- They are Gram-negative but are better stained with Giemsa and Dienes stains
- They differ from other bacteria in that they lack rigid cell wall and they are bound by a single trilaminar cell membrane that contains cholesterol or caratenol
- Some species show bulbous enlargement by means of which it attaches to receptor. They may be responsible for haemadsorption
- They multiply by binary fission, but genomic replication and cell division are often asynchronous so budding and chains of beads are formed

■ Describe the cultural characteristics of *Mycoplasma*.

Cultural Characteristics

- Aerobes and facultative anaerobes
- Optimum temperature for growth is 37°C
- Require sterol and cholesterol as essential growth factors

Culture Media

- ***Mycoplasma* broth:** It consists of bovine heart infusion broth, 20% horse serum, 10% fresh yeast extract, glucose and phenol red indicator
- ***Mycoplasma* agar:** *Mycoplasma* broth can be solidified by addition of agar. Penicillin, polymyxin B, ampicillin can be added to prevent growth of the contaminating bacteria and cycloheximide can be added to prevent growth of the contaminating fungi
- **Biphasic media:** The biphasic medium containing agar slant and broth of same composition can be used

Colony Characters

- Colonies usually appear after 2 or 3 days, but culture should be incubated for 4 weeks before final culture report
- Colony appears umbonate in reflected light and gives 'fried egg appearance' in transmitted light. Most colonies are haemolytic, about 200–500 μm in diameter
- Initially cell multiplies in agar to form a ball-shaped, opaque colony that grows up to the surface of agar and spreads around it forming translucent peripheral zone and it presents as 'Fried egg appearance'. Colony can be observed by hand lens
- Small colony, about 15–60 μm in size, is produced by *U. urealyticum* that lacks peripheral zone. Colonies cannot be picked up with a loop. Subcultures are done by cutting out agar block with colonies and rubbing it on a fresh plate
- Visualization of colony is facilitated by Dienes stain

Dienes stain

- Dienes stain is a mixture of methylene blue, maltose, benzoic acid, and sodium carbonate
- Method of staining*
 - Plate containing colony is flooded with stain (diluted 1:10 with distilled water)
 - It is then washed immediately with distilled water to remove excess stain
 - Medium is then decolorized by 95% ethanol
 - It is allowed to remain in contact for 1 minute and then removed
 - Plate is allowed to dry after washing
- Colonies are observed under low power objective
- Colony appears highly granular and stains with dark blue centre and light blue periphery
- Agar background appears clear and slightly violet
- *Mycoplasma* other than *M. pneumoniae* remain unstained
- *M. pneumoniae* reduces methylene blue after a period of time and colony becomes colourless

■ Mention the biochemical reactions that distinguish *Mycoplasma* species.

Biochemical Reactions

- Common feature: *Mycoplasma* species have fermentative properties
- Distinguishing features: Biochemical reactions specific to some species are as follows:

	Utilization of glucose	Arginine	Urea
<i>M. pneumoniae</i>	+	–	–
<i>M. hominis</i>	–	+	–
<i>U. urealyticum</i>	–	–	+

+ = utilized, – = not utilized.

■ To which agents are *Mycoplasmas* susceptible and to which are they resistant?

- *Mycoplasmas* are susceptible to heat
- They are susceptible to lysis by surface-active agents and lipolytic agents such as taurocholate
- They are resistant to penicillins and cephalosporins and lysozymes as they lack cell wall

■ Name two surface antigens present in *Mycoplasma*.

The two important surface antigens are:

1. Glycolipid antigen
2. Protein antigen

■ Describe infections caused by *Mycoplasma* spp. and *Ureaplasma* spp.

Mycoplasma and *Ureaplasma* are responsible for respiratory and urogenital infections.

Respiratory Infections

- *M. hominis* responsible for mild respiratory infections
- *M. pneumoniae* is responsible for **primary atypical pneumonia**
- Primary atypical pneumonia is also known as *Mycoplasma pneumoniae*
- It is characterized by prodromal features such as fever, malaise, headache, sore throat and respiratory symptoms such as cough, haemoptysis and chest signs
- In addition, it can cause pharyngitis, sinusitis and tracheobronchitis

Urogenital Infections

- Species mainly involved are *U. urealyticum* and *M. hominis*
- Diseases caused are nongonococcal urethritis, it can also cause balanitis, epididymitis and balanoposthitis in males
- In females, it causes acute salpingitis, PID, cervicitis and vaginitis
- It is also a cause of infertility, abortions, low birth weight babies and post-partum fever

■ Describe the procedures used in laboratory diagnosis of *Mycoplasma* infections.

Specimens

- **Respiratory**—sputum, throat swab, nasal swab, nasopharyngeal aspirate, broncho-alveolar lavage, tracheal aspirate
- **Genitourinary**—cervical swab, vaginal swab, semen, prostatic secretion, urine

Collection

Samples should be collected in Stuart's medium.

Transport

As mycoplasma is a delicate bacterium, samples should be inoculated immediately. If immediate culture is not possible, then specimen should be stored at 4°C. If delay of more than 24 hours is expected, sample should be frozen at -70°C. Standard Mycoplasma medium containing mixture of basal broth, bovine serum, albumen, gelatin and penicillin can be used as a transport medium.

Culture

Media used are:

1. Mycoplasma broth
2. Mycoplasma agar
3. Biphasic medium
4. Cell sheet

Mycoplasma Broth

In this glucose, phenol red and penicillin in addition to basal medium are used. Medium shows colour change from red to yellow and turbidity indicating growth of *M. pneumoniae* while other *Mycoplasma* show turbidity only because they do not utilize glucose.

Mycoplasma Agar

After 5–7 days of incubation, *M. pneumoniae* shows characteristic 'fried egg' colonies, which are large, 200–500 micron with central opaque area surrounded by transparent peripheral zone.

Colony—Identification

- Colonies of *Ureaplasma* are tiny about 15–50 μm in diameter and without peripheral zone
- Colonies of *M. pneumoniae* are identified by
 1. **Haemadsorption test:** In this test colonies show haemadsorption when plate is flooded with washed suspension of guinea pig RBCs, washed and incubated after washing
 2. **Tetrazolium reduction test (TTC):** This test is based on the ability of bacteria to reduce triphenyl tetrazolium chloride (TTC) to red compound formazan. In this test when colonies are flooded with TTC and incubated at 45°C for 1 hour, they appear reddish in colour and purple black after 4 hours
 3. **Serological technique:** This includes inhibition of colonies around the disc impregnated with specific antisera or fluorescence of colonies treated with antisera labelled with fluorochrome

Antigen Detection

Antigen can be detected in respiratory specimens by

1. Immunofluorescence
2. Counter-immunoelectrophoresis
3. Enzyme immunoassay
4. Immunoblotting with monoclonal antibodies

Nucleic Acid Detection

This method involves:

1. r-RNA detection by Gene-probe
2. DNA detection by dot-blot hybridization and polymerase chain reaction

Antibody Detection

Specific tests

1. Complement fixation test (CFT)—Significant titre in recent infection is 1:64 and antibodies appear after 7 days of infection
2. Indirect haemagglutination test
3. EIA for detection of IgM, IgG, IgA. The test is more sensitive than CFT

Nonspecific tests

1. Cold agglutination test—the test is based on appearance of a macroglobulin in patients of primary atypical pneumonia that agglutinate human “O” blood group RBCs at low temperature. Serial dilutions of sera are tested by adding equal volumes of RBCs and observed for clumping after leaving sera at 4°C overnight. Demonstration of rising titre or a titre of 1:32 is significant
2. *Streptococcus* MG test—serial dilutions of sera are tested by adding heat killed suspension of *Streptococcus* MG and observed after overnight incubation at 37°C. Titre of 1:20 or more is suggestive of infection

■ How can *Mycoplasma* infections be treated?

- Tetracyclines are effective
- Mycoplasmas are resistant to penicillins and cephalosporins

Miscellaneous Bacteria

■ What is HACEK group? Mention the important features of its representative species.

HACEK is a group of slow growing (7–30 days) fastidious bacteria of oropharyngeal or urogenital flora causing bacterial endocarditis.

H—*Haemophilus* species—*H. parainfluenzae*, *H. aphrophilus*

A—*Actinobacillus actinomycetemcomitans*

C—*Cardiobacterium hominis*

E—*Eikenella corrodens*

K—*Kingella kingae*

Important features of the species are as follows:

H. aphrophilus

- It is a species of the genus *Haemophilus*
- It requires X factor and not V factor for growth
- It needs high CO₂ concentration for growth
- It causes bacterial endocarditis

A. actinomycetemcomitans

- It is one of the species of the genus *Actinobacillus*
- It is a nonmotile, nonsporing, Gram-negative coccobacillus
- It grows better under anaerobic conditions
- On enriched media it forms small, rough colony that forms star-shaped or crossed cigar-like appearance. It does not grow on MacConkey's agar
- It is less active in fermenting sugars
- It is associated with actinomycotic lesions, abscesses and endocarditis

C. hominis

- It is a short, oxidase-positive, Gram-negative bacillus, which is nonmotile, nonsporing, noncapsulated
- It is fastidious aerobe and facultative anaerobe, requires CO₂ supplementation for growth
- On blood agar it forms small, glistening, circular colonies
- It is commensal in nose
- It can cause endocarditis in those with pre-existing cardiac disease and rarely meningitis

E. corrodens

- It is an oxidase-positive, capnophilic, Gram-negative bacillus
- It is commensal in upper gastrointestinal tract and respiratory tract
- It forms pitting or corroding of blood agar by colonies
- It causes infection of skin, subcutaneous tissue following salivary contamination
- It can also cause meningitis, endocarditis and pneumonia

K. kingae

- It is an oxidase-positive, Gram-negative nonmotile bacillus
- It is associated with endocarditis and infections of bone and joints

■ Write a short note on *Gardnerella vaginalis*.

- *Gardnerella vaginalis* is a Gram-negative bacillus that shows presence of metachromatic granules
- It is oxidase-positive and catalase-negative
- It grows on blood agar and chocolate agar forming minute colonies
- It is a causative agent of bacterial vaginosis, a condition characterized by foul smelling discharge of pH > 4.5 and presence of **clue cells** (epithelial cells with surface studded with numerous bacteria) in vaginal swabs
- This condition is usually associated with nonsporing anaerobic bacteria, particularly *Mobiluncus* spp.

■ Write a short note on *Listeria monocytogenes*.

- It is a small, Gram-positive coccobacillus usually arranged at angles
- It is catalase-positive
- It shows tumbling motility at 25°C in wet preparations and is nonmotile at 37°C
- It grows well on routine media and forms small, translucent haemolytic colonies on blood agar
- It shows umbrella pattern in semisolid motility medium
- Human infection is acquired through
 - Ingestion of contaminated food and milk
 - Contact with infected animal
 - Inhalation of contaminated dust
- It commonly causes disease in immunocompromised hosts and neonates but also in healthy pregnant women
- It can cause meningitis and meningoencephalitis in neonates and elder patients
- It can cause abortions, stillbirths and infertility
- It also causes abscess, conjunctivitis and endocarditis

■ Write a short note on *Erysipelothrix rhusiopathiae*.

- It is a Gram-negative filamentous rod
- It is nonmotile, nonsporing, noncapsulated
- It can grow on ordinary media
- It causes infection in animals, which are transmitted to humans by handling animal products, causing erysipeloid
- Lesions are usually on hands and fingers
- They are oedematous and erythematous, painful, and involve lymph nodes

■ Write a short note on *Calymmatobacterium granulomatis*.

- It is a Gram-negative coccobacillus also called *Donovania granulomatis*
- It shows bipolar or safety pin appearance
- It is capsulated and nonmotile
- Cultured on egg-yolk medium or on modified Levinthal agar
- It causes a venereal disease, donovanosis, which starts as a painless papule on genitalia and forms ulcer.
- Disease is diagnosed by demonstration of Donovan bodies in Giemsa stained smears
- Donovan bodies—*C. granulomatis* contained within the cytoplasmic vacuoles of large macrophages

■ **State the characteristics of *Acinetobacter calcoaceticus*.**

Characteristics of *Acinetobacter calcoaceticus* are, as follows:

- *A. calcoaceticus* is a strict aerobic Gram-negative coccobacillus
- It is oxidase—negative and a nonfermenter
- It occurs usually in pairs like *Neisseria*
- It is an opportunistic pathogen that causes hospital infections, particularly meningitis

■ **Write a short note on *Streptobacillus moniliformis* and *Spirillum minus*.**

- *S. moniliformis* is a Gram-negative, nonmotile bacillus and *S. minus* is short, motile, spiral, better visualized by Giemsa stain or dark ground microscope.
- These are causative agents of rat bite fever, characterized by fever, rash and arthralgia after rat bite.
- Disease is diagnosed by isolation of bacilli from blood and other body fluids
- Serological tests such as CFT, FAT, agglutination test are available for diagnosis

■ **Write a short note on *Campylobacter*.**

- *Campylobacter* are oxidase—positive, Gram-negative curved bacilli like *Vibrio* but are biochemically inactive
- There are more than 10 species
- Species of human importance are *C. coli* and *C. jejuni*
- *C. coli* causes mild enterocolitis and *C. jejuni* causes dysentery
- *C. jejuni* infection is acquired by faeco-oral route following ingestion of raw milk or contaminated water
- It acts by producing enterotoxin as well as by penetration of gut epithelium
- It causes a disease characterized by crampy abdominal pain, fever with chills and bloody diarrhoea
- It resembles *Vibrio* in morphology and motility
- It requires selective media for isolation, such as Butler's medium, Skirrow's campylobacter selective medium

■ **Write a short note on *Helicobacter*.**

- *Helicobacter* are spiral or helical bacteria
- They are strict microaerophilic
- Species of importance is—*H. pylori*
- *H. pylori* are short, spiral, Gram-negative bacteria, motile with tuft of sheathed flagella
- They get transformed to coccoid forms on exposure to air
- *H. pylori* are microaerophilic, need CO₂ supplementation and humid environment
- The species can grow on Skirrow's *Campylobacter* media and chocolate agar producing circular, convex, translucent colonies
- They are catalase, urease and oxidase-positive
- Strong urease reaction is their characteristic feature
- They cause chronic gastritis, duodenitis and peptic ulcers
- Tests for identification are biopsy, urease test and urea broth test

UNIT

IV

Virology

55

Chapter

General Properties of Viruses

■ What does virology deal with?

Virology is the branch of microbiology that deals with the study of viruses.

✓ What are viruses?

- Viruses are the smallest (20–300 nm in diameter), obligate intracellular infectious agents
- They do not have cellular organization
- Their genome is an element of nucleic acid, either DNA or RNA
- Their genome is enclosed in a protein shell known as **capsid**, which may be surrounded by a lipid-containing membrane called **envelope**
- The entire infectious unit is called "**Virion**"—an extracellular infectious virus particle (Fig. 55.1)

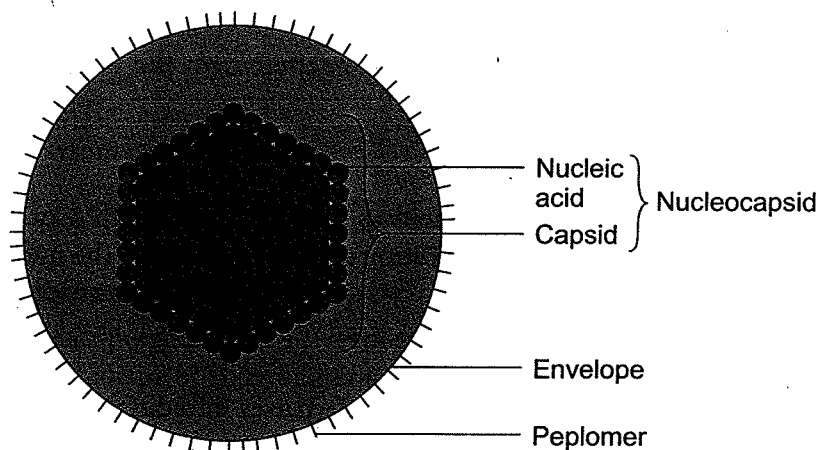


Fig. 55.1 Structure of virus.

✓ Differentiate between viruses and bacteria.

Features differentiating viruses and bacteria are presented in Table 55.1.

■ What is the size of viruses and how can it be measured?

- Viruses are ultramicroscopic particles that can be seen only under an electron microscope
- The size ranges from 20 nm to 300 nm in diameter
 - Largest virus: 300 nm, e.g. smallpox virus
 - Smallest virus: 20 nm, e.g. parvovirus
- The size of virus can be measured by
 - Direct observation under the electron microscope
 - Passing them through the filters of different pore size
 - Based on the rate of sedimentation in the ultracentrifuge

✓ **Table 55.1** Differences between viruses and bacteria

Viruses	Bacteria
1. Much smaller in size—measured in nm	Larger—measured in μ
2. Contain only one type of nucleic acid either DNA or RNA	Contain both DNA and RNA
3. Do not have a cellular organization and ribosomes	Possess cellular organization and ribosomes
4. Inert in extracellular environment	Active in extracellular environment also
5. Replicate only inside the living cells	Replicate outside the living cells also
6. Lack the enzymes necessary for synthesis of proteins and nucleic acid	Possess enzymes necessary for synthesis of proteins and nucleic acid
7. Obligate intracellular parasites not affected by antibiotics	Not obligate intracellular parasites and affected by antibiotics
8. Filterable through bacteriological filters because of their small size	Not filterable through bacteriological filters because of their large size

Describe the structure and chemical nature of a virus.

Structure and Chemical Nature

- A virus has two major components (Fig. 55.1):
 1. Nucleic acid—core or genome
 2. Protein sheath—capsid
- The nucleic acid core is surrounded by a protein coat known as **capsid**
- The protein nucleic acid complex—the capsid, together with the enclosed nucleic acid is known as the **nucleocapsid**

Nucleic Acid

- Viruses contain a single kind of nucleic acid as genome—either DNA or RNA—in which genetic information necessary for the replication of virus is present. The nucleic acid may be:
 - Single-stranded or double-stranded
 - Circular or linear
 - Segmented or unsegmented
- These properties form the basis of classification of viruses into different families

Protein Sheath (Capsid)

- It refers to protein shell that encloses nucleic acid (genome). It is composed of polypeptide chains called **protomers**
- Protomers contain a single polypeptide chain, which forms the basic structural unit
- The protomers aggregate into groups of 5 or 6 to form structures called **capsomeres**
- The capsid is formed by a number of capsomeres

Functions

- Protection to the nucleic acid by forming an impermeable shell around it
- Transfer of nucleic acid from one host cell to the other
- Promotes attachment to the susceptible cell and its receptors

Envelope

- A number of viruses possess a membrane covering surrounding the nucleocapsid. It is known as **envelope**

- It is about 10–15 nm thick and is acquired from the nuclear or cytoplasmic membrane of the host cell during replication process
- It is lipoprotein in nature. The lipids are derived from the host cell and the proteins are virus coded
- In some viruses, e.g. myxoviruses, the proteins (glycoproteins) are present as projections called **spikes** or **peplomers** about 10 nm long
- Enveloped viruses are susceptible to ether and other organic solvents
- Treatment with ether results in loss of lipids—leads to loss of infectivity

Functions

- Envelope confers antigenic, biological and chemical properties on viruses
- The peplomers help to attach virus particle to a host cell by interacting with a cellular receptor (Fig. 55.1)

■ **What shapes of viruses are generally observed?**

- The shape is different in different groups of viruses
- Viruses may be of the following shapes:
 - Brick-shaped, e.g. poxvirus
 - Bullet-shaped, e.g. rabies virus
 - Rod-shaped, e.g. tobacco mosaic virus
 - Irregular and pleomorphic in some viruses

■ **Which are the three types of symmetry observed in viruses? Briefly explain the structure of each type and give examples.**

- The symmetry varies according to the number of capsomeres and their arrangement
- Three types of symmetry are determined based on the arrangement of capsid around the nucleic acid core. These are as follows:
 1. Icosahedral symmetry
 2. Helical symmetry
 3. Complex symmetry

Icosahedral Symmetry

- An icosahedron is a polygon with 12 vertices or corners and 20 facets or sides (Fig. 55.2)
- Each facet is in the shape of an equilateral triangle

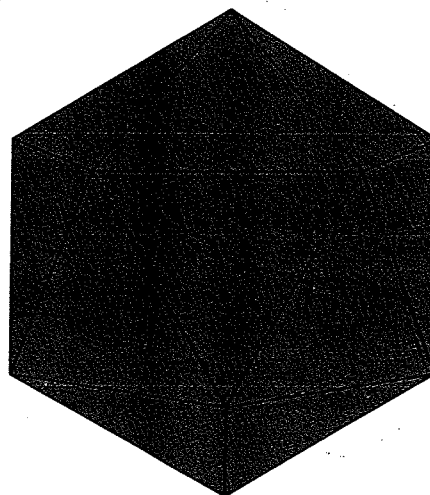


Fig. 55.2 An icosahedron

- It has a rigid structure
- Two types of capsomeres—pentons and hexons—constitute the icosahedral capsid
- Pentons are present at the vertices and hexons make up the facets
- Icosahedron symmetry is found in:
 - DNA viruses—such as herpesvirus, parvovirus, papovavirus and adenovirus
 - RNA viruses—such as picornavirus and reovirus

Helical Symmetry

In this, the protein subunits (capsomeres) are packed in the helical array around the viral nucleic acid to form a spiral tube or helix (Fig. 55.3)

- The tube may be rigid, e.g. tobacco mosaic virus (TMV) or flexible in many animal viruses
- Helical symmetry is found in RNA viruses and most of them are enveloped viruses, such as myxoviruses, arboviruses, TMV and rhabdoviruses. It is unsuitable for DNA viruses

Complex Symmetry

- Some viruses have a very complicated structure, which is neither icosahedral nor helical
- These are referred to have complex symmetry, e.g. poxviruses (Fig. 55.4)

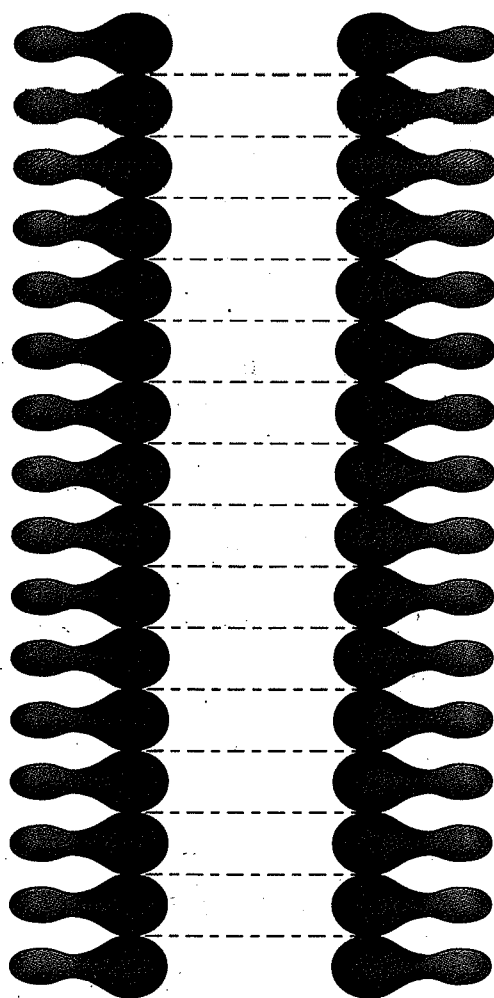


Fig. 55.3 Helical symmetry.

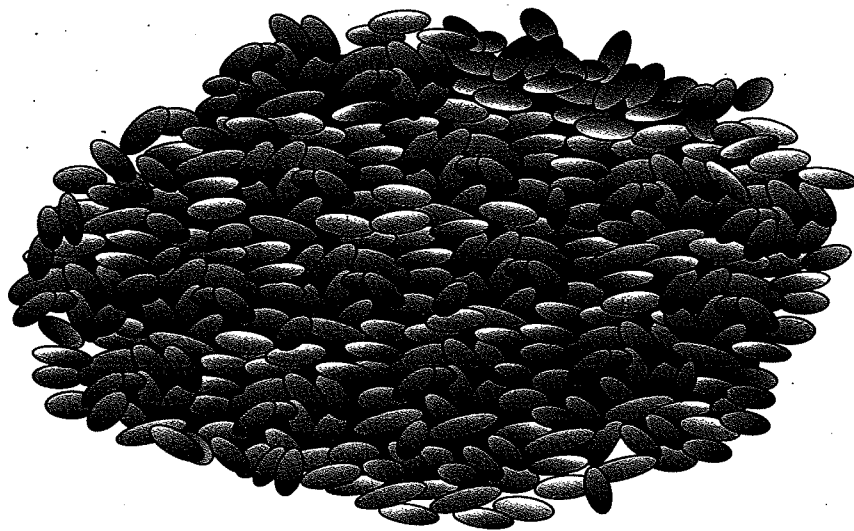


Fig. 55.4 Complex symmetry.

■ **Discuss susceptibility of viruses to physical and chemical agents.**

- Viruses are sensitive to heat—inactivated by heating at 56°–60°C in 30 minutes; exceptions are Hepatitis B virus and polio virus
- Viruses are resistant to cold and can survive –70°C
- pH range 5–9, but viruses are destroyed by extreme acidity or alkalinity
- Viruses are sensitive to oxidizing agents such as hydrogen peroxide, potassium permanganate and organic iodophores
- Enveloped viruses containing lipids are sensitive to ether
- Some viruses are sensitive to phenols and cresols
- Betapropiolactone is actively virucidal
- Glutaraldehyde is effective against viruses and has an important role in the disinfection of apparatus used in renal dialysis units
- Formaldehyde is active but slow in action
- Chlorination of water kills most viruses but hepatitis A and polio viruses are relatively resistant to chlorination, especially when present with organic or faecal material
- Viruses are sensitive to sunlight, ultraviolet rays and ionizing radiations
- Viruses are resistant to antibiotics and chemotherapeutic agents

■ **Describe the proteins, lipids and carbohydrates found in viruses.**

Viral Proteins

Two types of proteins are present in viruses, viz.

1. Structural proteins—proteins present in the **coat of virus**
2. Nonstructural proteins—these are functional proteins such as **haemagglutinins** and **enzymes**

Haemagglutinins

- These are proteins present as haemagglutinin spikes (peplomers) on the envelope, which can agglutinate erythrocytes of various animal species
- These are glycoproteins, which have special affinity for glycoproteins located on the surface of RBCs (receptor areas)
- When RBCs are added to serial dilutions of viral suspension, the virus interacts with RBCs, adhere to each other causing haemagglutination

- This test is used as simple and rapid method for detection of viruses in egg or tissue culture fluid
- The haemagglutination reaction can be specifically inhibited by the antibody to the virus—this is known as haemagglutination inhibition test—used for detecting antiviral antibody in diagnosis and research

Viral enzymes

Viruses produce different types of enzymes. These include:

1. Neuraminidase
2. RNA polymerase
3. Reverse transcriptase
4. Enzymes of cellular origin

Neuraminidase

- It is an enzyme present as peplomer on the surface of some viruses (influenza and parainfluenza viruses). Also produced by many bacteria including *V. cholerae*
- It acts on the receptors on RBCs and destroys them and hence known as receptor-destroying enzyme (RDE)
- It causes destruction of surface receptors and reversal of haemagglutination and release of viruses from the surface of RBCs. This process is known as elution
- It causes irreversible damage to receptors and makes RBCs nonagglutinable by that virus

Viral Lipids

The lipids are derived from host cell membrane and hence their composition is similar to host cell membrane.

Viral Carbohydrates

Carbohydrates are present in nucleic acids—as a part of them—in the core as glycoproteins (e.g. poxviruses), on the surface as glycoproteins and in the outer capsid proteins (e.g. rotaviruses).

■ Explain the viral haemagglutination test.

Viral Haemagglutination Test

- It is agglutination of RBCs caused by viruses possessing haemagglutinin spikes
- It is a simple and rapid method used for detecting viral growth in culture

Procedure

- RBCs are placed in tubes or plastic trays
- Serially diluted viral suspension is added
- Observed for the highest dilution-causing haemagglutination to determine titre after incubation at 4°C or 37°C

Result

- Positive test: Agglutinated RBCs are seen spread into a shield-like pattern
- Negative test: Nonagglutinated RBCs settle at the bottom in the form of a button (Fig. 55.5)
- Titre: Highest dilution showing positive test provides the titre

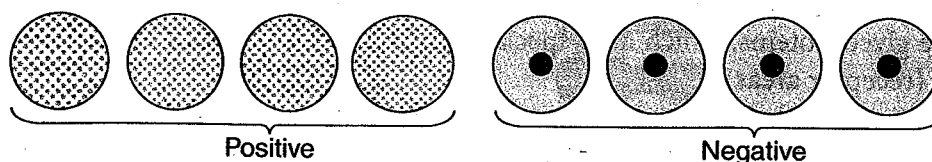


Fig. 55.5 Viral haemagglutination.

- As the inactivated virus can also haemagglutinate, the test is also used to titrate killed influenza virus
- RBCs are used in haemagglutination tests
- For influenza, parainfluenza and mumps virus—fowl, man and guinea pig RBCs (cause elution at 37°C) are used—for Toga, rubella, and rabies—goose RBCs at 4°C
- For enteroviruses and reoviruses—human RBCs at 37°C
- For measles—monkey RBCs at 37°C.

Describe the process of viral replication.

Replication of Viruses

For an animal or plant, cell is the fundamental unit of structure and function. For virus, the cell is merely a means of making new virus particles. As viruses lack enzymes necessary for biosynthesis, they utilize the biochemical machinery of the host cell to synthesize virus specific macromolecules. The host cell not only provides the energy and biosynthetic machinery but also provides the low molecular weight precursors for synthesis of viral proteins and nucleic acid required for the production of virus progeny.

The viral nucleic acid carries the genetic information necessary for viral replication. As soon as it enters the cell, the cellular metabolism is redirected exclusively towards the synthesis of new viral particles and replication occurs in sequential phases. The different phases in virus replication are (Fig. 55.6):

1. Adsorption
2. Penetration

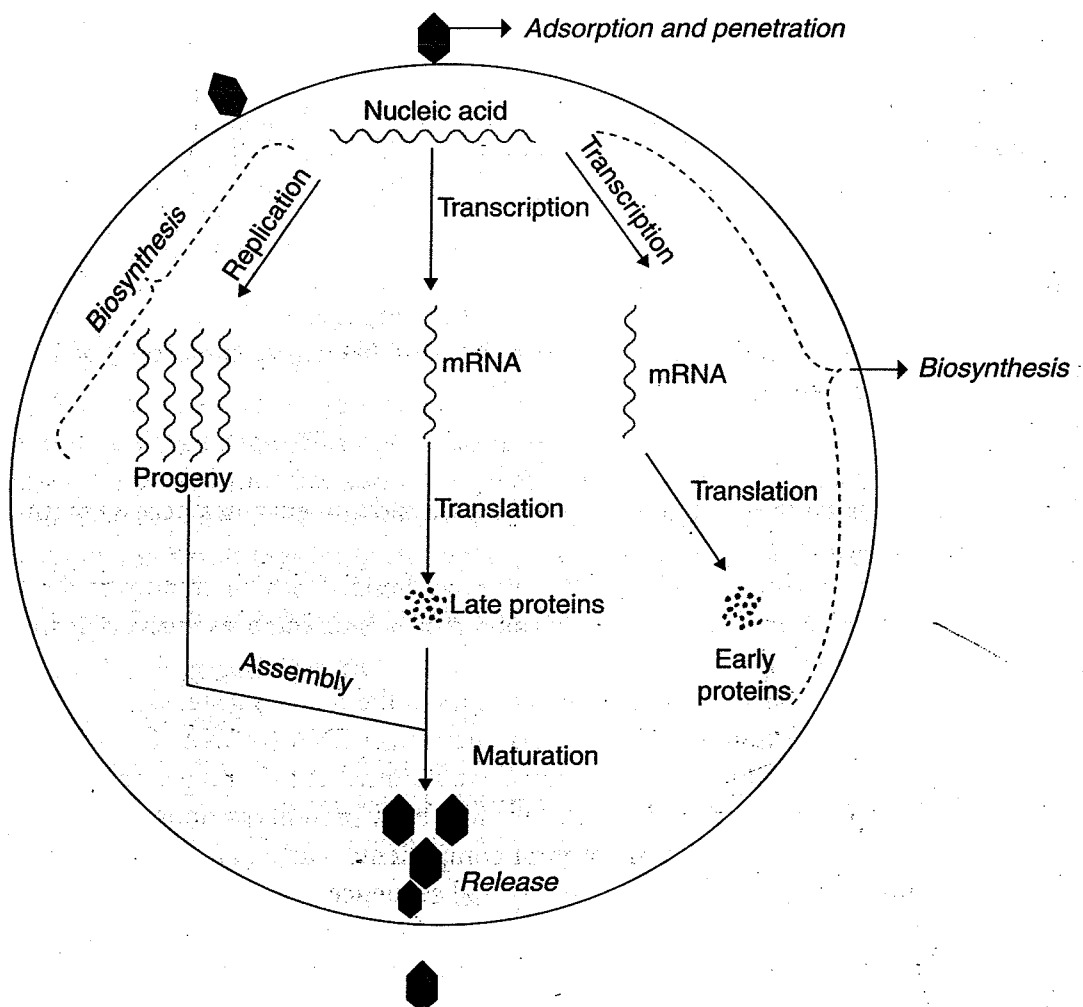


Fig. 55.6 Replication of viruses.

3. Uncoating
4. Biosynthesis
5. Maturation
6. Release

Adsorption or Attachment

- The first step in viral infection is attachment. A highly specific interaction of virus with receptors on host cell surface results in attachment of virus to the host cell
- Virus may come in contact with cell by random collision, but adsorption occurs only if there is an affinity between them. For this, the cell surface must possess receptor sites, which are complementarily fit for virus adsorption structures known as **ligands**
- The nature of these ligands is different in different viruses, e.g.
 1. In HIV, the surface glycoprotein—gp 120 is the ligand that binds to CD4 receptor on T cells
 2. In adenoviruses, each pentamer at the corner of the capsid bears a small fibre that serves as a **ligand**
 3. In enveloped viruses—the spikes on the surface act as ligands, e.g. in influenza virus, the haemagglutinin spike—glycoprotein in nature—binds to receptor sites on respiratory epithelium

Penetration

It is the taking of virus particle inside the cell. This occurs in the following ways:

1. It occurs by a process called **viropexis** or **endocytosis** in which the host cell membrane forms a vesicle around the virus particle, which detaches from the cell membrane and carry the virus particle deep into the cytoplasm
2. The virus may be directly admitted through the plasma membrane, e.g. adenovirus
3. In case of enveloped virus, there is fusion of virus envelope with plasma membrane of the host cell releasing the nucleocapsid into the host cytoplasm

Uncoating

- It is removal of capsid and release of nucleic acid into host cell
- This is a physical separation of the viral nucleic acid affected by the action of lysosomal enzymes of the host cell

Biosynthesis

- This phase includes synthesis of viral nucleic acid, capsid and enzymes necessary in various stages of viral replication
- In addition, certain regulatory proteins are also synthesized, which shutdown the normal cellular metabolism so that the cellular metabolism is redirected exclusively towards the synthesis of viral components
- In both DNA and RNA viruses, biosynthesis occurs in the following steps:
 - First step is **transcription**, i.e. formation of messenger RNA (mRNA) from viral nucleic acid. Specific encoded information is transferred to mRNA by viral genome
 - The second step is **translation** of the mRNA into early proteins or nonstructural proteins (enzymes) necessary for synthesis of viral components. Particular sequence of bases in mRNA forms proteins with specific amino acid sequence
 - The third step is **replication** of viral nucleic acid. In general, the synthesis of nucleic acid occurs in the nucleus of the host in case of DNA viruses; however, it occurs in the cytoplasm of the host cell in case of RNA viruses. Exceptionally, the synthesis of genome can

- occur partly in nucleus and partly in cytoplasm, e.g. orthomyxoviruses, some paramyxoviruses and retroviruses
- The last step is **synthesis of late protein** or structural proteins (capsid protein); it occurs in the cytoplasm of the host cell

Maturation

- It consists of two processes—assembly and its association with nucleic acid
 - **Assembly:** The polypeptide chains (late proteins) are rapidly assembled into capsomeres to form procapsid
 - **Association with nucleic acid:** The procapsid finally incorporates nucleic acid and becomes a complete virion—the daughter virus
- The process of maturation may take place in the cytoplasm or the nucleus of the host cell
- In herpes and adeno viruses, it occurs in the nucleus. In this case, the polypeptides are transported to the nucleus where they are incorporated into capsid
- In picorna and poxviruses, it occurs in the cytoplasm
- The enveloped viruses acquire envelope through the process of budding. In herpes virus, the envelope is derived from the nuclear membrane as the maturation occurs in nucleus. However, in other viruses like orthomyxo and paramyxoviruses, in which maturation occurs in cytoplasm, it is derived from plasma membrane

Release

It occurs by three mechanisms. These are as follows:

1. **By cell lysis:** Nonenveloped viruses are released through rupture in the cell membrane—cell lysis occurs because of the large number of virus particles in the host cell. This follows the death of the host cell
2. **By budding:** Enveloped viruses are released by this mechanism. This process takes longer time for release but does not kill the host cell. In this method, the nucleocapsid moves towards the cell membrane and binds to it. The cell membrane curves around it and releases the virus by budding
3. **By cell degeneration:** Viruses such as parvoviruses accumulate in the host nucleus and are released after the cell death, which follows degeneration of the cell

■ What is the 'eclipse phase' in the growth cycle of a virus?

- Eclipse is the phase of the growth cycle that includes the period starting from the stage of penetration of virus into host cell to the appearance of first infectious virus progeny particle. During this period the virus cannot be demonstrated inside the host cell
- Its duration depends upon the infecting virus and the host cell
- It is about 15–30 minutes for bacteriophages and 15–30 hours for animal viruses

■ Describe the abnormal replicative cycles in viruses.

Abnormal Replicative Cycles

Incomplete Viruses

Defective assembly of virus particles may lead to formation of incomplete daughter viruses that may not be infective, e.g. formation of defective influenza virus with high haemagglutination titre but low infectivity. This is known as **von Magnus Phenomenon**

Abortive Viral Infections

- Infection of host cell by a virus is not always productive resulting into formation of large number of viruses

- Sometimes, the viral components may not be synthesized, or if synthesized, may not be properly assembled
- The virus progeny either is not released or is noninfectious
- Such processes are known as abortive infections
- These may be due to defect in the host cell, which is not able to synthesize the products as per the demands of the virus or because of some inherent fault in the virus itself

Defective Viruses

- Some viruses, which are unable to replicate themselves and unable to give rise to fully formed progeny because of genetic defect are known as defective viruses
- These can replicate in the presence of helper viruses, e.g. hepatitis D virus and adeno-associated viruses, which replicate in the presence of hepatitis B virus and adenoviruses, respectively

Pseudovirions

- Sometimes, instead of viral nucleic acid, host cell nucleic acid is enclosed by capsid
- Such particles are noninfective, nonreplicating and are called pseudovirions

LSN ■ **How are viruses cultivated? Give a detailed description of each method.**

- As viruses are obligate intracellular parasites, they can be grown in living cells only
- Three types of methods have been developed for their cultivation. These are:
 1. Animal inoculation
 2. Embryonated eggs
 3. Tissue culture

Animal Inoculation

This was the first method used to grow viruses but it has slowly disappeared because of its disadvantages such as (a) interference of immunity with viral growth, (b) infection of animal with latent viruses, (c) high cost, (d) difficulty in handling (e) maintenance of animals, (f) laws regarding experimental use of animals and (g) availability of other simpler methods. **However, animals are still used for:**

1. Primary isolation of viruses, which do not grow in eggs and tissue cultures
2. To study pathogenesis of viral diseases
3. To study immune response
4. To study efficacy of vaccines and drugs
5. To study oncogenesis

Animals commonly used

- Suckling mice—most common
 - Rabbits
 - Guinea pigs
 - Monkeys
 - Hamsters
 - Ferrets
- } In some viruses

Route of inoculation

- Intracerebral
- Intraperitoneal
- Intranasal
- Subcutaneous

Indicator of growth

- Disease or visible lesions
- Death
- Testing of presence of virus in tissue by neutralization test using antiviral sera
- Demonstration of inclusion bodies

Embryonated Eggs

- Good Pasture in 1931 first used embryonated hen's egg for cultivation of viruses
- The embryonated eggs have several advantages over animals. These include:
 - Simple to handle
 - Cost effective and easily available
 - No immunological interference with growth
- Embryonated hen's eggs, usually 7–12 days old are inoculated by one of the following routes (Fig. 55.7):
 1. Chorioallantoic membrane (CAM)
 2. Allantoic cavity
 3. Amniotic cavity
 4. Yolk sac

After inoculation, eggs are incubated for 2–9 days based on the type of virus and route of inoculation.

Chorioallantoic membrane (CAM)

- Some viruses can grow on CAM, producing visible lesions called pocks
- The morphology of pock is different with different viruses
- As each virus particle forms one pock, the number of pocks indicates number of virus particles in the inoculum. Thus, it helps in the assay of pock-forming viruses
- Used for isolation, diagnosis and assay of poxviruses and herpes simplex virus

Allantoic cavity

- Viruses such as influenza and some paramyxoviruses are grown in allantoic cavity
- It is mainly used for growing viruses such as influenza, yellow fever and rabies virus for vaccine production, because of high yield of virus in this cavity
- The growth of virus is detected by haemagglutination test

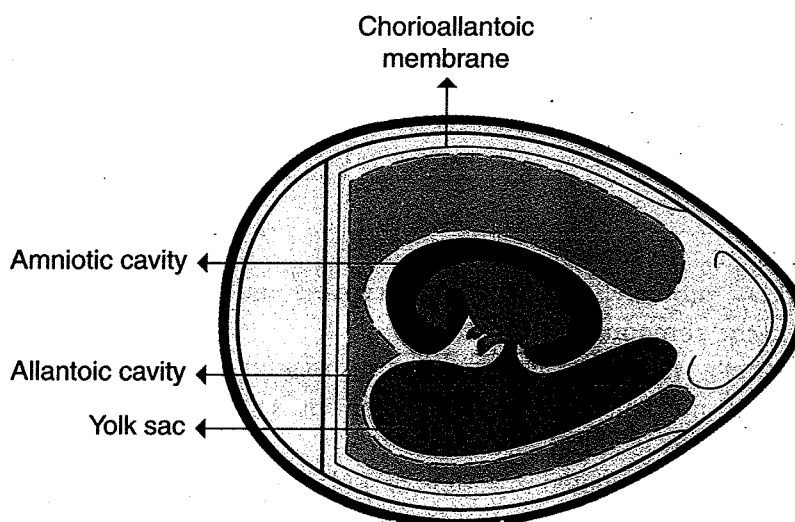


Fig. 55.7 Embryonated egg—Routes of inoculation.

Amniotic cavity

- It is mainly used for the primary isolation of influenza virus
- It can also be used for obtaining high yield of virus for vaccine production
- The growth of virus is detected by haemagglutination test

Yolk sac

It is used for cultivation of some viruses, e.g. Rabies virus and bacteria such as chlamydiae and rickettsiae.

Indicators of growth

- Pock formation on CAM by pock-forming viruses
- Detection of haemagglutination titres in amniotic cavity and allantoic cavity

Disadvantages of egg inoculation

1. It does not permit a controlled study of virus-cell interaction
2. Primary isolation of many viruses is not possible in egg—fail to grow on primary inoculation

Tissue Culture (Cell Lines)

Three types of tissue cultures are available. These are:

1. Organ culture—whole organ
2. Explant culture
3. Cell culture—most commonly used

Organ culture

Small bits of organs maintained *in vitro* in a tissue culture growth medium are used for isolation of highly specialized viruses of certain organs, e.g. coronavirus isolation using tracheal ring culture

Explant culture

Fragments of minced tissue—grown as explants embedded in plasma clots, e.g. adenoid tissue explant culture for isolation of adenoviruses. Rarely used nowadays.

Cell culture

- Most commonly used—usually in monolayers
- Monolayers are produced from desired tissues

Preparation of Monolayers

- Desired tissue is cut into small pieces
- By treating with a proteolytic enzyme (e.g. trypsin) and by mechanical shaking, tissue is dissociated into the component cells
- The dissociated cells are washed, counted and suspended in a suitable growth medium containing all essential nutrients (amino acids, vitamins, salts, glucose, etc.) and supplemented with up to 5% of calf or fetal calf serum
- The cell suspension is distributed in a glass or a plastic container
- On incubation, the cells adhere to glass or plastic surface and divide to form a confluent monolayer sheet of cells within a week

ST
TV

Ba
car

1
2
3

Pr

•
•
•

Us
Th

Di

1
2
3

Di

•

•

Us

1

2

Cc

•

•

•

•

•

•

Ex

1

2

Types of Cell Culture

Based on their origin, chromosomal characters and number of generations through which they can be maintained, cell cultures are classified into three types. These are:

1. Primary cell cultures
2. Diploid cell cultures
3. Continuous cell lines

Primary Cell Cultures

- These are normal cells—freshly taken from the organs of animal or human beings and cultured
- These are capable of only 5–10 divisions at the most, i.e. the growth is limited
- They cannot be maintained in serial cultures, e.g.
 1. Rhesus monkey kidney cell culture
 2. Human amnion cell culture
 3. Chick embryo fibroblast cell culture

Uses

The method is used for the primary isolation of viruses and their cultivation for vaccine production.

Disadvantages

1. Limited number of cell divisions—short life
2. High cost
3. Animal tissue may contain latent viruses

Diploid Cell Strains

- These are cells of a single type that retain the original diploid chromosome and karyotype during serial subcultivation for a limited number of times. It is based on the lifespan of the species of the animal from which it is obtained
- The human diploid cells undergo around 50 divisions, e.g.
 1. WI-38—Human embryonic lung cell strain
 2. HL-8—Rhesus embryo cell strains

Uses

1. Diploid cell developed from human fibroblasts are susceptible to a number of human viruses and are useful for the isolation of some fastidious pathogens
2. They are also used for commercial production of vaccine, e.g. WI-38 for rabies vaccine

Continuous Cell Lines

- These are immortal cell lines capable of indefinite growth *in vitro*
- They are usually derived from cancerous tissue or originate by spontaneous transformation of a diploid cell strain
- These cells grow faster and their chromosomes are haploid
- These cells can be maintained indefinitely by serial subcultures at regular intervals or stored at -70°C for use when necessary and when large numbers of cells are required they can be grown in suspension culture
- These cell lines are derived from monkeys, dogs, cattle, pig, cat, mouse, hamster, rabbit and human beings

Examples

1. HeLa—Human carcinoma of cervix cell line
2. HEP-2—Human epithelioma of larynx cell line

3. KB—Human carcinoma of nasopharynx cell line
4. McCoy—Human synovial carcinoma cell line
5. Detroit-6—Sternal marrow cell line
6. Chang C/I/L/K—Human conjunctiva (C), intestine (I), liver (L) and kidney (K) cell line
7. BHK-21—Baby Hamster kidney cell line
8. Vero—Vervet monkey kidney cell line

Uses

1. Isolation of viruses from clinical specimens
2. For production of antigens
3. Some cell lines are used for vaccine production, e.g. vero cell line for rabies vaccine

■ Describe the techniques employed for detecting viral growth in cell cultures.

The growth of virus can be detected by the following techniques:

Cytopathic Effects (CPE)

- Many viruses kill the cell in which they grow while many others cause detectable morphological changes known as CPE. The viruses that causing CPE are known as cytopathogenic viruses
- The CPE are different in different groups of viruses and help in the presumptive identification of virus isolates

Some viruses produce detectable CPE, however, others produce CPE, which require visualization under the microscope

- CPE induced by viruses are as follows:
 1. Rounding of cells—nuclear pyknosis, rounding, ability to refract, and degeneration in picornavirus group
 2. Syncytium formation—fusion of infected cell with adjacent cells to form giant cells containing several nuclei, as in measles and respiratory syncytial viral infections
 3. Rounding and aggregation of cells into grape-like clusters in adenoviruses
 4. Crenation of cells and degeneration of the entire cell sheet in enteroviruses
 5. Formation of intracytoplasmic or intranuclear inclusion bodies

Haemadsorption

- The growth of haemagglutinating viruses such as myxoviruses and Togaviruses can be detected by adding guinea pig RBCs to the cell cultures
- The adsorption of RBCs to the surface of cells indicates multiplication of viruses. No adsorption indicates absence of viruses
- This phenomenon is known as haemadsorption

Interference

- The growth of noncytopathogenic virus in cell culture can be detected by subsequent inoculation (challenge) of a known cytopathogenic virus
- No growth of known cytopathogenic virus indicates growth of a noncytopathogenic virus
- The noncytopathogenic virus, if present, inhibits growth of cytopathogenic virus by interference

Immunofluorescence

- The growth of virus in infected cells can be detected by staining with specific antiviral antibody conjugated with a fluorescent dye
- This method gives rapid results, earlier than other methods, hence used to identify many viral isolates

Detection of Enzymes

The growth can be ascertained in some viruses by detecting viral enzymes in the culture fluid, e.g. detection of reverse transcriptase in retroviruses.

Electron Microscopy

Viruses grown in culture can be demonstrated by observing under an electron microscope.

Transformation

The growth of oncogenic viruses can be detected by the transformation of cultured cells.

■ Describe the various types of viral assays used for analysis of viral infections.

Two types of viral assays are used:

1. Total virus particle count
2. Infectious virions assay

Total Virus Particle Count

This can be achieved by the following two methods:

1. Electron microscope

- This is the most sensitive and ideal method
- The number of viral particles in a suspension can be counted directly under the electron microscope by using
 - Negative staining with potassium phosphotungstate
 - Mixing with latex particles.

2. Haemagglutination assay

- Viruses causing agglutination of RBCs can be quantitated by determining the haemagglutination titres
- This is a crude method not very sensitive but it is simple
- It requires about 10^7 influenza virus particles to produce haemagglutination
- This is not a good method for assay when less than 10^7 virus particles are present

Infectious Virions Assay

The following two types of assays are used:

1. Quantitative Assay

It measures the number of viable infectious particles in the suspension. Two methods are in use. These are:

- (a) **Plaque assay in monolayer cell culture:** In this method, viral suspension is inoculated onto monolayer in a bottle or Petri dish. After giving sufficient time for adsorption of the virus to the cells, the monolayer is covered with agar gel to restrict the spread of virus to adjacent cells. Each virus particle gives rise to a localized focus of infected cells that becomes visible to the naked eye and is known as plaque. Each plaque indicates an infectious virus. The infectivity titre is expressed in terms of plaque forming units (PFU) per millilitre
- (b) **Pock assay in eggs:** Viruses that form pocks on CAM (e.g. poxviruses) of the chick embryo can be assayed by counting the number of pocks. As each viable infecting particle gives rise to one pock, the number of pocks formed indicates number of viruses in the inoculum
- (c) **Transformation assay:** For oncogenic viruses

2. Quantal Assays

- These assays indicate the presence or absence of infectious virus and do not measure the number of infectious particles in the inoculum
- In these assays, serial dilutions of the virus suspension are inoculated into animals, eggs or tissue culture medium
- Death of inoculated animal, haemagglutination in allantoic cavity or the CPE in cell culture is considered as end point for infectivity titration
- The virus titre is expressed as the ID₅₀ (50% infectious dose)/ml
- The ID₅₀ indicates the highest dilution of the inoculum that would produce an effect in 50% of animals, eggs or tissue culture inoculated

■ Classify viruses on the basis of the type of nucleic acid and morphological characters.

Classification of Viruses

- Viruses are classified on the basis of physiochemical and biological properties into families, which are split into genera and finally into species
- The important criteria employed are:
 - Type of nucleic acid of the genome and strategy of viral replication
 - Morphological characters such as size, shape, structure and presence of envelope
- Classification is primarily based upon type, polarity and shape of the nucleic acid
- Viruses are broadly categorized as DNA and RNA viruses and further subdivided on the basis of other characters (Table 55.2)

■ How is the system of nomenclature designed for naming viruses?

Nomenclature of Viruses

- Viruses are grouped into families based on nucleic acid of the genome
- Families are named with a suffix—*viridae*
- Families are subdivided into subfamilies, which are named with suffix *virinae*
- Subfamilies are subdivided into genera, which are named with suffix *virus*

Table 55.2 Classification of viruses

Class	Nucleic acid	Envelope	Shape	Example
RNA viruses				
Ia	(+), ss RNA	Absent	Icosahedral	Picornaviruses
Ib	(+), ss RNA	Present	Icosahedral	Togaviruses
II	(-), ss RNA	Present	Helical	Paramyxoviruses
III	(-), ss RNA segmented	Present	Helical	Orthomyxoviruses, Arenaviruses
IV	ds RNA, segmented	Absent	Icosahedral	Reoviruses
V	(+), ss -RNA	Present	Helical	Retroviruses
DNA viruses				
Ia	ds linear DNA	Absent	Icosahedral	Adenoviruses
Ib	ds linear DNA	Present	Icosahedral	Herpesviruses
Ic	ds linear DNA	Present	Complex	Poxviruses
II	ds circular DNA	Absent	Icosahedral	Papovaviruses
III	ds linear DNA	Absent	Icosahedral	Parvoviruses

ss = single-stranded, ds = double-stranded.

- The prefix may be a Latin word or a sigla—an abbreviation derived from some initial letters
- The latinized names are written in italics and vernacular names are written in roman letters

■ **What are viroids?**

- Viroids are plant pathogens, which do not exhibit any extracellular dormant phase
- They contain single-stranded RNA molecules without protein coat (capsids)
- They are resistant to heat but sensitive to nuclease
- They do not cause human infections

■ **Write a short note on prions.**

- Proteinaceous infectious particles without any detectable nucleic acid
- Do not exhibit virus morphology (being viruses without nucleic acid genome) under electron microscope
- Highly resistant to physical and chemical agents
- Sensitive to proteases but resistant to nucleases
- Multiply in cell culture but unable to produce cytopathic effects (CPE)
- Do not elicit Ab formation and inflammatory reaction
- Difficult to transmit
- These are the cause of slow infections with long incubation period (in years) such as spongiform encephalopathies in animals (mad cow disease) and humans

56

Chapter

Virus-Host Interactions (Virus Infection)

■ What are the effects of viral infections expressed in the host? Which cellular mechanisms are involved in causing cell injury?

- Interaction of virus with host may cause various effects, ranging from no apparent cellular damage to rapid cell destruction, e.g.
 - Cytocidal or cytolysis—poliovirus
 - Cell proliferation—*Molluscum contagiosum*
 - Malignant transformation—oncogenic viruses
 - Latent infection—herpes simplex virus
 - Steady state infection—without any cellular injury
 - Morphological change in cells to form inclusion bodies—rabies virus
- The mechanism by which cellular injury is produced is different in different viruses. The mechanisms of cell damage include:
 - Shutting down of protein and DNA synthesis of host cell by early proteins
 - Accumulation of viral macromolecules causing distortion of cellular architecture and toxic effects
 - Alteration of permeability of plasma membrane causing release of lysosomal enzymes and autolysis
 - Alterations in the cytoplasmic membrane of infected cells, e.g. fusion of adjacent cell membranes leading to syncytium formation or expression of virus coded Ags on the surface of infected cells conferring new properties
 - Damage to the chromosome of host cell, e.g. mumps, measles, adenovirus, etc.
 - Formation of inclusion bodies

■ What are inclusion bodies? How are they useful in identifying viral infections?

- These are virus-specific intracellular globular masses that are produced during the replication of virus in host cells
- These are structures with distinct size, shape location and staining properties, which can be demonstrated in virus-infected host cells under the light microscope
- The inclusions may be:
 - Cytoplasmic
 - Intranuclear, or
 - Both cytoplasmic and intranuclear
- **Cytoplasmic (intracytoplasmic) inclusions** are produced by viruses that assemble in the cytoplasm. These are mainly produced by RNA viruses, e.g.
 - Negri bodies by rabies virus
 - Guarnieri bodies by vaccinia virus
 - Molluscum bodies (large 20–30 μ) by *Molluscum contagiosum*
 - Bollinger bodies by fowlpox virus

- **Intranuclear inclusions** are produced by viruses that assemble in the nucleus. These are usually produced by DNA viruses. These are of the following two types:
 1. Cowdry type A—are of variable size and granular appearance, e.g. herpes and yellow fever virus
 2. Cowdry type B—circumscribed and often multiple, e.g. adenovirus and poliovirus
- **Both cytoplasmic and intranuclear inclusion bodies**—are produced by measles virus (RNA virus)
 - They are generally acidophilic, stain pink when stained with Giemsa or eosin methylene blue stains. Some are basophilic, e.g. adenovirus inclusion bodies and are stained by haematoxylin
 - They may be crystalline aggregates of virions or made up of virus antigens present at the site of virus synthesis
 - Their demonstration helps in the diagnosis of some viral infections, e.g. Negri bodies in the cytoplasm of brain cells help in the presumptive diagnosis of rabies

✓ Mention the routes by which viruses gain entry into the host. Cite examples.

The various routes by which viruses may enter the host are as follows:

- **Mucous membranes**—such as
 - Conjunctival sac, e.g. some adeno- and enteroviruses
 - Nose and throat, e.g. influenza, rhino, mumps, measles, rubella, varicella-zoster virus (VZV), cytomegalovirus (CMV), etc.
 - Gastrointestinal, e.g. hepatitis A virus, poliovirus, rotavirus, etc.
 - Genital, e.g. human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), herpes simplex virus (HSV), etc.
- **Skin**—entry of viruses by way of skin occurs through
 - Abrasions—papilloma, *Molluscum contagiosum*
 - Insect bites—arboviruses
 - Animal bites—rabies virus
 - Injections—HBV, HIV, etc.
- **Subcutaneous and muscular tissue**—virus enters by insect and animal bite or by injection, e.g. rabies virus, arboviruses
- **Placenta**—congenital transfer of virus from mother to fetus, may occur through placenta, e.g. rubella virus and cytomegalovirus cross the placenta and may lead to abortion, maldevelopment or severe neonatal disease. This transmission of virus from mother to fetus is known as **vertical transmission**

✓ SN Briefly describe the methods by which virus spreads in the host.

After entry in the host, virus may spread locally causing localized infections or may cause generalized infections spreading via blood or lymph and subsequent localization in target organ. The spread of virus occurs in the following ways:

1. Direct Cell to Cell Spread

Viruses may spread directly from cell to its neighbouring cells by forming intracellular bridge, e.g. in warts (skin cells), common cold (mucosal cells) and in conjunctival infections.

2. Role of Macrophages

Viruses resistant to the phagocytic activity of macrophages, if ingested, grow inside the macrophage and spread to various parts along with phagocytic mobility.

3. Role of Lymphatic System

- After entry through skin, subcutaneous tissue or mucous membrane, viruses may reach the regional lymph nodes

- If immune mechanisms fail to overcome infection in the node, they may be carried in the lymph and to the blood stream and finally to the target organ

4. Role of Blood and Vascular System

- After local multiplication in lymph node, virus may enter the bloodstream causing **primary viraemia**
- It is then transported to the different organs
- The spleen and liver are the central foci for viral multiplication, where extensive multiplication of virus occurs that leads to a massive spillover of the virus into bloodstream causing **secondary viraemia**
- Alternatively, virus may reach the circulating blood with the help of leucocytes in the tissues, which ingest viruses and make their way into the blood through the walls of blood vessels
- In blood, virus may be associated with granulocytes, monocytes, RBCs or platelets (cell-associated viraemia) or may move freely in plasma (plasma viraemia)

5. Spread by Nerve Fibres

Neurotropic viruses such as herpes, rabies and arboviruses can spread along the nerve fibres.

■ What is tropism?

- Viruses have selective affinity for one or more target organs in the body. This property is known as **tropism**
- The skin and central nervous system are the main target organs. Liver, lungs, muscles, abdominal viscera, mucous membrane and heart may also get infected
- Based on tropism, viruses may be classified as dermatropic, neurotropic, pneumotropic, enterotropic or hepatotropic
- Tropism is genetically determined by the property of the virus, which is host-specific, age-specific and organ-specific

✓ ■ What is meant by incubation period? What determines the duration of incubation period in viral infections? Cite examples.

- Incubation period (IP) is the interval between the first entry of infective virus and the first onset of symptoms. It includes the time from entry to spread to the target organs to produce lesions and symptoms
- IP duration is influenced by the relation between the site of entry, target organ, multiplication rate and lesions
- **Examples:** IP—short: 1 to 3 days—when site of entry and site of multiplication are same, e.g. respiratory viral infections
 - IP—long: 10–20 days—when site of entry and site of multiplication are different, e.g. systemic diseases like poliomyelitis
 - IP—in HBV may be 2–6 months and in slow viruses may be many years

■ In what possible ways do viruses bring about damage to host tissue, eliciting infection?

- Once the defense mechanisms of the host are overcome, the virus can initiate damage to host tissues producing a mild, severe or fatal illness
- The damage to host tissue is produced probably in two different ways, viz.
 - **Direct cytotoxicity**—by viral components and virus specified products. These are termed **viral cytotoxins**
 - **Immunopathological reactions**—damage to virus infected host cell may be produced by type II (antibody-mediated) or Type IV (cell-mediated) cytotoxic reactions. Uninfected

host cells may also be damaged by the immune response evoked by host to viral antigens or by Type I (anaphylaxis) and Type III (immune complex) reactions

- This damage may result in
 - Death of host cell
 - Proliferation of host cell—formation of multinucleated giant cells by fusion of membranes of adjacent cells
 - Malignant transformation of host cell

✓ **When is virus released from the host?**

Release of virus from the host is the final stage in pathogenesis

- Viruses are released from host in the last few days of incubation period, during the acute phase of illness and only for short period thereafter
- Chronic release of viruses as in tuberculosis does not occur
- However, in some viral diseases, virus may persist in a latent phase after the initial or primary infection, which may be stimulated to resume infective condition and cause release of virus, e.g. herpesvirus, cytomegalovirus, etc.
- The viruses are released from the site of local multiplication, such as upper and lower respiratory tract, gastrointestinal tract, genitourinary tract, etc.

✓ **Discuss in detail the host response to viral infections?**

The host response to viral infections may be

1. Specific immune response, or
2. Nonspecific response

Specific Immune Response

It is of the following two types:

1. **Antibody-mediated or humoral immunity:**

- IgG, IgM and IgA produced in response to virus infection offer protection against viruses
- IgG and IgM play major role against viruses invading blood and tissue spaces
- IgA is produced locally and is selectively concentrated in seromucous secretions of respiratory, intestinal and urogenital tracts. It offers major protection at local level and acts as barrier to prevent systemic spread of viruses from localized surface areas
- Abs may act in the following ways:
 - Neutralize the viruses and prevent their attachment, penetration and uncoating
 - Attach to viral Ags on the surface of infected cells, making these cells prone to lysis by complement or destruction by phagocytic cells or killer (K) cells
- Cause immune opsonization of virus and phagocytosis

2. **Cell-mediated immunity (CMI):**

- It is primarily mediated by T lymphocytes, which have virus-specific receptors on their surface, with the help of which they respond to viruses
- The cells other than T cells such as NK cells, killer cells and macrophages directly attack the viruses
- The different mechanisms of action are as follows:
 - T lymphocytes (cytotoxic T cells) are directly cytotoxic to virally infected host cells
 - Lymphokines released by sensitized T cells encourage macrophages to discourage the intracellular bridge formation and limit the intracellular transfer
 - NK cells attack on virally infected host cells and cause cytolysis
 - Killer cells attack virally infected host cells with the help of Abs (Ab-dependent cell-mediated cytotoxicity)

- There may be Ab- and complement-mediated cytotoxicity
- T cells produce interferon, which interferes with multiplication of virus and limit the infection

Nonspecific Response

These include many important physiological defenses, which are as follows:

1. **Phagocytosis:** Macrophages play important role in clearing viruses from the bloodstream. Polymorphonuclear leucocytes do not play any significant role
2. **Body temperature:** As most viruses are inhibited by temperature above 39°C, fever acts as natural defense against virus infections
3. **Interferons** (a family of glycoproteins): These are antiviral substances produced by cells in response to viral infection and also in response to nonviral microorganisms
 - Interferons (IFN) were isolated, characterized and named so by Isaacs and Lindenmann (1957)
 - Interferons have antiviral activity by inhibiting proteins synthesis
 - They act by conferring on cells resistance to multiplication of number of different viruses
 - Interferons are not virus specific; hence interferons produced in response to one virus can confer protection against infection by same or unrelated viruses. However, some interferons have species (host) specificity, e.g. mouse interferons are ineffective in human and vice versa

Types of Interferons—these are of the following three types:

1. IFN alpha—produced by leucocytes in response to virus infection. It has antiviral activity
2. IFN beta—produced by fibroblasts and epithelial cells in response to virus infection. It has antiviral effect
3. IFN gamma—produced by T lymphocytes and NK cells in response to antigens and mitogens. It is a lymphokine that has modulatory/regulatory effect on immune system. It enhances MHC antigens and activates cytotoxic T cells, macrophages and NK cells

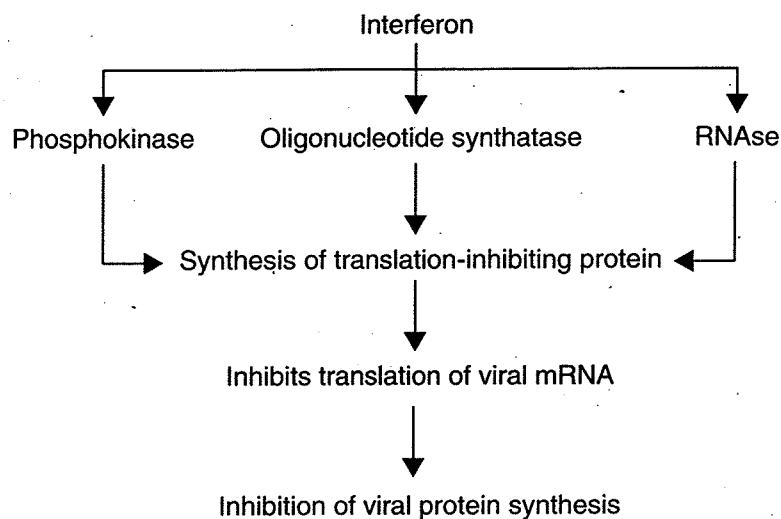
■ How does interferon inhibit spread of viral infection within a host?

- Interferon by itself has no direct action on viruses and is not active in the cell in which it has been induced
- It moves out of the cell in which it is produced and acts on neighbouring cells by interacting with specific receptors at the cell surface
- This interaction triggers the synthesis of cellular protein (translation-inhibiting protein-TIP) that selectively inhibits viral mRNA, thereby inhibiting viral protein synthesis. Viral transcription may also be inhibited
- This protein is a mixture of three different enzymes, namely phosphokinase, oligonucleotide synthetase and RNase, which ultimately cause inhibition of protein synthesis and render neighbouring cells refractory to virus infection
- Mechanism of action of interferon is summarized in Flowchart 56.1

■ Enumerate the properties of interferons.

Properties of Interferons

- Protein in nature (molecular weight, 20,000–40,000)
- Inactivated by proteolytic enzymes but not by nuclease or lipases
- Resist heating at 56°–60°C for 30–60 minutes and pH range of 2–10, except IFN gamma, which is labile at pH 2
- Nondialysable and nonsedimentable
- Poorly antigenic and species specific



Flowchart 56.1 Role of interferon in inhibiting viral infection.

■ **Mention the agents that induce synthesis of interferons.**

- Many substances induce synthesis of interferon
- **Viruses** vary in their capacity to induce interferon

Examples

1. Cytocidal and virulent viruses are poor inducers
2. Avirulent viruses—good inducers
3. RNA viruses better than DNA viruses
4. Natural and synthetic double-stranded RNA—more efficient
5. RNA and DNA animal viruses, plant viruses—good inducers
6. Intracellular organisms like-bacteria, rickettsia, protozoa
7. Bacterial products such as lipopolysaccharide, polysaccharide

✓ **What biological effects are produced by interferons?**

Biological effects of interferons are as follows:

- **Antiviral activity**—inhibits replication of virus
- **Antimicrobial activity**—against intracellular pathogens such as toxoplasma, *Chlamydia*, malarial parasites, etc.
- **Immunoregulatory effects**—enhance cytotoxic activity of macrophages, NK, K and T cells. Modulation of Ab formation, activation of Ts cells and suppression of delayed hypersensitivity
- **Antitumour activity**—inhibits growth of tumour cells by tumour necrosis factor (TNF) like cytokine
- **Cellular effects**—inhibition of cell growth and proliferation, inhibition of DNA and protein synthesis and increased expression of MHC Ags on cell surfaces

■ **Mention the therapeutic applications of interferons.**

Interferons are used in the treatment of

- **Viral infections**, e.g. herpes keratitis, genital warts and respiratory diseases
- **Benign tumours**, e.g. laryngeal papilloma
- **Malignant tumours**, e.g. breast cancer, osteosarcoma, multiple myeloma and other cancers

Discuss immunoprophylaxis of viral diseases.

- On account of obligate intracellular parasitism of viruses, only few drugs are available, which are useful against viral infections. Hence, prevention of infection by using vaccines is very important
- Four types of vaccines are available—live vaccines, killed vaccines, subunit vaccines and recombinant vaccines.

Live Vaccines

Contain live virus with reduced virulence. They initiate infection without causing disease, and hence offer greater and long-lasting protection

Preparation

Live vaccines are prepared by using naturally attenuated strain or they are attenuated by cultivating the virus serially into various hosts or cultures. Alternatively, vaccine strains with desired antigens can be developed by genetic techniques, e.g. influenza virus by DNA recombinant technology

Advantages

1. A single dose is usually sufficient as virus multiplies in the host, producing a prolonged antigenic stimulus
2. They can be administered by the route of natural infection to induce local immunity—both IgA and IgG are produced when the vaccine is administered by natural route of infection. They also induce cell-mediated immunity
3. Induces a long-lasting immunity, hence booster doses are generally not required
4. They are more economical and convenient for the administration

Disadvantages

1. Risk of reversion of virulence as it is composed of attenuated viral mutants
2. Virus may spread from vaccinee (vaccinated person) to contact (one who can acquire infection). This is a double-edged sword. It is advantageous if the spread immunizes others and dangerous if virulent revertant spreads to a susceptible person
3. Interference by pre-existing virus, if present, may prevent a good immune response
4. They are heat-labile; hence, proper storage conditions are necessary

Killed Vaccines

They contain killed viruses. They do not initiate infection like live vaccines, hence immunity developed is less and also the protection lasts for short duration

Preparation

They are prepared by inactivation of virus by heat, phenol, formalin or beta-propiolactone.

Advantages

1. Safe and stable
2. No risk of spread of virus from vaccinee to contacts
3. Can be given in combination as polyvalent vaccine

Disadvantages

1. Immunity induced lasts for short duration and offers less protection
2. Usually administered by parenteral route (oral route is not suitable), hence do not stimulate a major IgA response (local immunity)

3. CMI is also not induced
4. Multiple injections are needed (booster doses)
5. Extreme care is required to ascertain the potential problem of inadequate inactivation
6. May sometimes cause hypersensitivity reactions

Subunit Vaccines

These are preparations, containing relevant antigens from viruses, which are immunogenic components. These vaccines include rabies vaccine and influenza vaccine

Recombinant Vaccines

In these vaccines, the genes responsible for specific antigens, which are immunogenic component from viruses, are cloned in organisms like *E. coli*, Vaccinia virus or other suitable vectors and then the antigen is obtained in large amount. The technique is known as DNA recombinant technology. These vaccines include:

- Vaccine against hepatitis B virus
- Influenza vaccine

Passive Immunization

Passive immunization using human gamma globulin, convalescent serum or specific antiserum gives temporary protection against many viral diseases, e.g. measles, mumps, infectious hepatitis, etc.

■ How are viral infections checked?

- As viruses are strict intracellular parasites, it is difficult to obtain selective toxicity against viruses. Despite this difficulty, viruses may be destroyed by attacking the vital processes of its growth cycle
- Virus infection may be checked at various steps of replication. These include:
 - Attachment and penetration
 - Transcription of viral nucleic acid
 - Translation of mRNA (viral protein synthesis)
 - Replication of viral nucleic acid
 - Assembly and release of virus

■ Mention the inhibitors of attachment and penetration.

The following are the inhibitors of attachment and penetration:

1. **Amantadine (Adamantanamine):** It inhibits the attachment of influenza virus and prevents entry of virus
2. **Rimantadine:** It is a derivative of amantadine, has similar effect and less side effects

■ Describe the various inhibitors of viral nucleic acid synthesis.

The following are the inhibitors of viral nucleic acid synthesis:

1. **Acyclovir:** It is a nucleoside analogue primarily active against HSV-1 and 2, and varicella-zoster virus. It acts by inhibiting virus encoded thymidine kinase. Topical application is effective in the treatment of primary genital herpes, herpes keratitis and herpes infections in patients undergoing immunosuppressive therapy
2. **Ganciclovir:** It is a nucleoside analogue of guanosine, structurally similar to acyclovir but more active against cytomegalovirus
3. **Vidarabine:** It is a nucleoside analogue that is effective against HSV-1

4. **Iododeoxyuridine:** It is a nucleoside analogue that inhibits replication of viral nucleic acid. It is too toxic to be used systemically. Clinically useful in topical therapy of herpetic keratoconjunctivitis
5. **Trifluorothymidine:** It is a nucleoside analogue too toxic for systemic use but useful in the topical treatment of herpetic keratoconjunctivitis
6. **Foscarnet:** It acts by inhibiting DNA polymerases of all herpes viruses and also inhibits the reverse transcriptase in HIV
7. **Azidothymidine (AZT, Zidovudine, Retrovir)**—it is a nucleoside analogue. It inhibits DNA synthesis by the enzyme reverse transcriptase. It is effective against HIV and is drug of choice for AIDS
8. **Dideoxyinosine (Didanosine):** Its action is similar to that of AZT. It is used as substitute for AZT in AIDS patients who are intolerant or resistant to AZT
9. **Dideoxycytidine (Zalcitabine):** Its action similar to that of AZT. It is used as substitute for AZT
10. **Ribavirin:** It is a nucleoside analogue. It acts by inhibiting the synthesis of guanine nucleotides. Used in the form of aerosol to treat pneumonitis caused by respiratory syncytial virus in infants and to treat severe influenza B virus infections. It inhibits many DNA and RNA viruses

■ **Mention the inhibitors of viral protein synthesis, the step that is targeted by them and an example of viral infection treated by use of such inhibitors.**

Inhibitors of viral protein synthesis are as follows:

1. **Interferon:** It inhibits protein synthesis by inhibiting translation of viral mRNA. It is useful in the treatment of chronic hepatitis B and C infections
2. **Methisazone:** It inhibits synthesis of late proteins by blocking translation of late mRNA. It is effective against poxviruses

57

Chapter

Laboratory Diagnosis of Viral Diseases

✓ What methods are employed for diagnosing viral diseases?

The methods used in the diagnosis of viral diseases are:

- Direct demonstration of virus and its components
- Isolation of viruses—can be isolated and identified during the course of disease. Isolation is difficult and time-consuming
- Detection of specific antibodies

■ Why is serology preferred over isolation of viruses for the purpose of diagnosis? Under what circumstances is isolation of viruses performed?

- Isolation and identification of viruses is routinely impracticable because of technical difficulties (difficulty in preparation of media, processing of specimen, inoculation, identification, etc.), length of time required, and lack of specific therapy for viral infections. Hence, serological tests are preferred
- Virus isolation is, however, required when
 - New epidemic occurs with new strain, e.g. in influenza
 - It is necessary to confirm presumptive diagnosis made by direct microscopy, e.g. in case of herpes virus
 - Same clinical illness may be caused by different agents
 - It is of vital importance, e.g. rubella in pregnancy
 - It is essential to define the cause of vague syndromes such as upper respiratory tract infection or aseptic meningitis
 - Detection and prediction of epidemics is essential
 - Antigenic variation in viruses is to be identified
 - Serological tests do not give any clue

✓ What types of specimens are suited for diagnosing viral infections?

- Specimens should be collected during the acute phase of the disease
- Appropriate specimens should be collected depending on the site of infection, e.g. sputum in respiratory infections
- The specimens collected include:
 - **In respiratory tract infections**—throat swab, nasal swab, nasal washing, nasopharyngeal aspirates, sputum, etc.
 - **In gastrointestinal tract infections**—stool and rectal swab. Stool is also collected in enterovirus infection of respiratory tract
 - **In central nervous system infections**—faeces, blood, CSF, brain biopsy, throat swab, rectal swab, saliva, etc.
 - **In skin infections**—macular/papular scrapings, vesicular/pustular fluid, ulcer scrapings, crust, faeces, etc.

- In ophthalmic infections—conjunctival scrapings or swabs
- In urinary tract infections—urine
- In HIV, hepatitis B, C and D virus infections—blood is collected

■ **What measures should be taken to ensure safe transport of specimens to a laboratory for diagnosis?**

- Specimen should be transported immediately to laboratory to avoid
 - Death of delicate viruses (many viruses are labile)
 - Overgrowth of bacteria and fungi
- If delay is expected, specimen should be transported in Stuart's viral transport media
- Blood for viral culture is collected and transported in a sterile vial containing anticoagulant. Blood can be stored at 4°C until processed and can be stored for months at -20°C or below

■ **List the methods of direct microscopy employed for demonstrating viruses.**

The different microscopic methods used for demonstrating viruses are as follows:

- Demonstration of cytopathic changes in infected cells, e.g. Tzanck cells in herpes simplex virus
- Demonstration of inclusion bodies in a stained smear under the light microscope (Giemsa or eosin methylene blue stains)
- Demonstration of virus by electron microscope or by immunoelectron microscopy
- Demonstration of virus in lesions by direct immunofluorescence test

✓ ■ **Name the methods by which viral antigens are demonstrated.**

When virus Ag is abundantly present in the lesions, it can be demonstrated/detected by serological methods such as

- Precipitation in gel (immunodiffusion)
- Immunofluorescence test
- Counter current immunoelectrophoresis (CIEP)
- Radioimmunoassay (RIA)
- Enzyme-linked immunosorbent assay (ELISA)
- Immunoperoxidase staining

✓ ■ **Mention the methods of detecting nucleic acid of viruses.**

Detection of viral nucleic acid can be performed by

- Nucleic acid probes (DNA probe analysis)
- Polymerase chain reaction (PCR)
- Reverse transcriptase PCR

■ **Mention the ways of ensuring contamination-free inoculation. Name the methods generally used for cultivating viruses.**

✓ **Preparation of Inocula**

Prior to inoculation, care is taken that inocula is free of bacteria. To achieve this:

- Specimens free from bacteria (CSF, blood, plasma or serum) are inoculated directly
- Specimens containing bacteria (throat swab, stool, sputum, tissue, etc.) are treated with antibiotics (if not harmful to viruses) to inactivate bacteria or filtered to remove bacteria or subjected to differential centrifugation to make inoculum free from bacteria

Inoculation

The inoculum is inoculated into

- **Animals**—such as
 - Suckling mice
 - Rats
 - Monkeys
 - Chimpanzees
- **Embryonated eggs**—the various sites are
 - Chorioallantoic membrane
 - Allantoic cavity
 - Amniotic cavity
 - Yolk sac
- **Tissue culture**—e.g.
 - Organ culture
 - Explant culture
 - Cell culture (primary, diploid, continuous)

■ Once isolated, how can viruses be identified?

The isolates are identified by using appropriate methods of identification depending on the culture method followed.

Identification of Virus in Experimental Animal

Viruses cultivated in experimental animals are identified by the following means:

- Analyzing characteristic lesions/disease/death
- Ab testing
- Demonstration of Ag in lesions
- Biopsy and autopsy studies

Identification of Virus Growth in Eggs

Viruses cultivated in eggs are identified by the following means:

- Haemagglutination titre
- Pock formation
- Inclusions

✓ Identification of Virus Growth in Tissue Culture

Methods of identification employed for tissue culture growth are:

- Studying characteristic cytopathic changes induced by virus
- Demonstration of virus under the electron microscope or by immunoelectron microscopy
- Demonstration of viral Ag by direct immunofluorescence test
- By using the following tests:
 - Neutralization test
 - Haemadsorption test
 - Haemagglutination test
 - Haemagglutination inhibition test
 - Cytopathogenic effects interference test
 - Metabolic inhibition test
 - Interference

- **Explain broadly the procedure for serological test. List the various types of serological tests generally adopted for identifying viruses.**

Procedure for Serological Tests

- Serial samples of blood are collected to demonstrate rise in titre. In general, first sample should be collected as soon as possible after the onset of the illness and second sample after 2 or 3 weeks
- Demonstration of a rise in titre of Abs to a virus during the course of illness is considered as a strong evidence, hence examination of paired sera is essential and demonstration of Abs in single sample may not be meaningful
- If paired sera are not available, a presumptive diagnosis can be made by demonstrating IgM Abs to the virus

Types of Serological Tests

The serological tests performed would depend on the virus, but in general the following tests are carried out:

- Neutralization test
- Complement fixation test
- Haemagglutination inhibition test
- Passive haemagglutination test
- Indirect immunofluorescence test
- Enzyme linked immunosorbent assay test
- Agar gel diffusion test
- Countercurrent immunoelectrophoresis test
- Radioimmunoassay
- Western blot test

58

Chapter

Bacteriophages

✓ **What are bacteriophages?**

Bacteriophages are viruses that infect and parasitize bacteria. These are usually called phages.

They are distributed widely in the environment—commonly present in sewage, faeces, polluted water, soil and other natural sources of mixed bacterial growth.

■ **Briefly narrate the events that led to the discovery of bacteriophages.**

- Twort (1915) described an infectious agent that produced a degenerative change in the culture of *Staphylococcus*
- d'Herelle (1917)—observed the lytic properties of filtrates of faeces cultures from dysentery patients—he found lysis of a broth culture of a dysentery bacillus

He suggested that the lytic agent was a virus and named it as bacteriophage (phage means 'to eat'), meaning bacteria eater.

✓ **Describe the structure of a bacteriophage.**

Bacteriophage is made up of the following components:

- **Phage capsid:** The phage genome is surrounded by a protein covering known as phage capsid
- The large phages, e.g. T-even phages (phages affecting *E. coli* are called T-even phages), are tadpole-shaped and possess a head and a tail
 - **Head:** It is hexagonal in shape. It encompasses the genome—double-stranded DNA enclosed in a capsid. The size of head varies in different phages from 28–100 nm
 - **Tail:** Cylindrical, composed of hollow core surrounded by a contractile sheath and a terminal base plate. Plate has attached pins or tail fibres or both (Fig. 58.1)
- **Genome:** Most phages contain a single, linear, double-stranded DNA molecule as genome.
- **Morphological variations**
 - Some phages are spherical or filamentous in shape
 - Some of them have single-stranded DNA as genome and some of them have RNA as their genome, e.g. phages attacking male strains of *E. coli*, contain RNA as genome

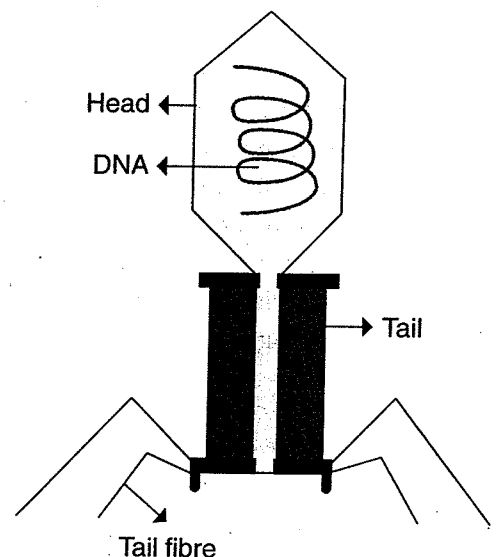


Fig. 58.1 Morphology of T-even phage.

■ Mention the important characteristics of bacteriophages.

Important characteristics of phages are enumerated below:

- Host specificity—they have high host specificity
- They are filterable through filters, which hold back the bacteria
- Lytic phages cause lysis of bacteria
- They are sensitive to heat—inactivated by boiling
- Their commonest habitat is intestinal bacterial flora of man and animal

■ Briefly explain the life cycle of bacteriophages.

Depending on its type, upon entering the host a bacteriophage can pass through either of the following two types of life cycles:

1. Lytic Cycle

In this, intracellular multiplication of phages occurs that results in the lysis of the host bacterium and release of progeny virions. This cycle is also known as **virulent cycle**.

2. Lysogenic Cycle

In this, there is integration of phage DNA into the bacterial genome that replicates with bacteria without causing any harm to the host cell. This cycle is also known as **temperate cycle**.

■ Describe the lytic life cycle of a virulent bacteriophage.

Lytic Cycle

Lytic cycle of phages passes through different phases, which are as follows:

Adsorption

- It is the first step, during which phages come in contact with bacterial cells by random collision and attach to the specific receptor site on bacterial cell surface with the help of complementary chemical groups on the receptor site on the terminal base plate of the phage
- It is a specific process, which occurs rapidly (within minutes) under optimal conditions

Penetration

- Following adsorption, the base plate and tail fibres are held firmly against the bacterial cell causing the hollow core to inject the phage DNA through cell walls
- The process of penetration resembles injection through a syringe that keeps the empty head (capsid) and tail outside the bacterial cell as the shell or ghost
- Penetration may be facilitated by the lysozymes present on the tail, which produce a hole on the bacterial cell wall for entry of phage DNA
- When a bacterial cell is attacked by large number of phages, multiple holes are produced on the cell causing lysis of cell without the viral multiplication. This is known as "**lysis from without**"

Synthesis of Phage Components

- After the penetration of DNA, synthesis of phage components is initiated
- Initially, early proteins, which include specific enzymes, necessary for synthesis of phage components, are synthesized. During this phase, the phage components are not detectable and this phase is known as **eclipse phase**. The duration of this phase is about 15–30 minutes
- After the eclipse phase, the synthesis of late proteins, which are protein subunits of phage head and tail, starts

Maturation

During this, the protein subunits of phage head and tail and phage DNA assemble to form the mature infective particle.

Release of Progeny Phages

- The release of phages occurs by lysis of the bacterial cell. The bacterial cell bursts (ruptures/undergoes lysis) releasing progeny viruses because of weakening of cell wall by phage enzymes (lysozyme) and because of osmotic pressure. This is called "**lysis from within**" (Fig. 58.2)
- The interval between the infection of a bacterial cell and the first release of phage particles is known as the **latent period**

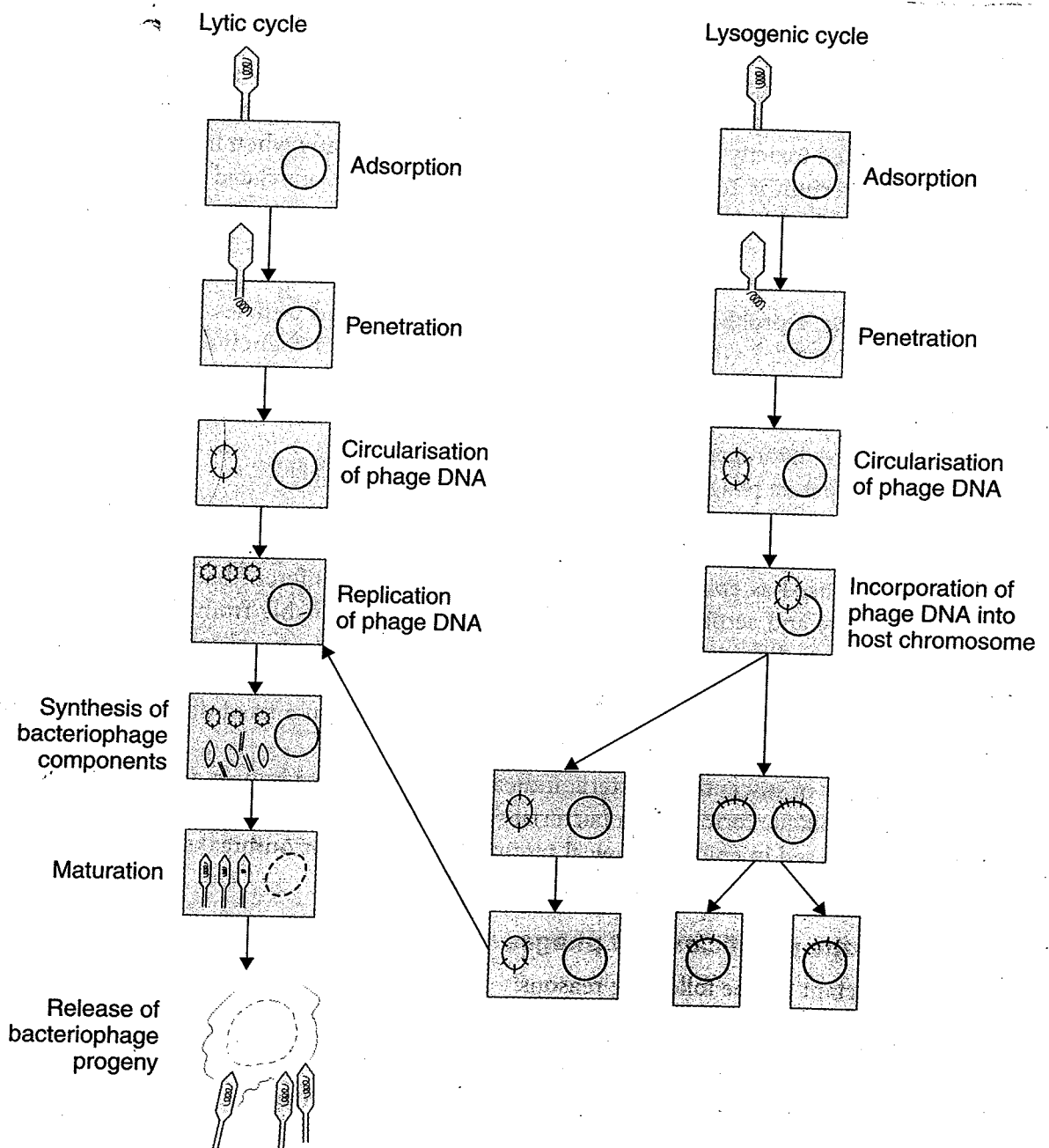


Fig. 58.2 Lytic and lysogenic cycle of bacteriophage.

■ Describe the lysogenic life cycle of a temperate bacteriophage.

Lysogenic Cycle

- Infection with phages does not always result in lytic cycle. Some phages after infection insert their nucleic acid (genome) into the bacterial chromosome
- The integrated phage genome in bacterial chromosome is known as **prophage**, which behaves like a segment of the host chromosome and replicates synchronously with it. This phenomenon is known as **lysogeny**
- The bacterium that carries prophage integrated into its chromosome is known as a **lysogen** or **lysogenic bacterium** and phage that persists in bacterium without causing lysis of bacterium is also known as **lysogenic** or **temperate phage** (Fig. 58.2)

■ What is lysogenic conversion?

- The prophage confers certain new properties on the lysogenic bacteria. This is known as **lysogenic conversion** or **phage conversion**. Some examples are:
 1. Phage-mediated toxigenicity of *C. diphtheriae*—exotoxin production in this organism is mediated by beta phage. Elimination of beta-phage makes the organism nontoxic
 2. Phage-mediated toxicity in *C. botulinum*—produces toxin only when infected by phages
- A lysogenic bacterium is resistant to reinfection by the same or related phages. It is also known as **superinfection immunity**

■ What is meant by 'spontaneous induction of prophage'?

- On rare occasions, a prophage excises from bacterial DNA and initiates lytic cycle. Forms daughter phage particles, which infect other bacteria and render them lysogenic
- This is known as spontaneous induction of prophage
- It is a very rare event and may be induced by physical (ultraviolet rays) and chemical agents (nitrogen mustard, hydrogen peroxide)
- Infection by temperate phage may also lead directly to lytic cycle

■ Describe bacteriophage typing.

- Phage typing is used as epidemiological marker to identify different bacterial strains that are biochemically and serologically identical and indistinguishable from each other. Such bacterial strains are differentiated by using strain or type-specific bacteriophages
- For this, the strain to be typed is inoculated on an agar plate to produce—lawn culture. The suspension of phages are then applied on a lawn culture in a fixed dose and incubated overnight
- The result is lysis of susceptible bacteria indicated by a clear area known as plaques
- On the basis of plaque formation, a bacterial species can be divided into various types
- Phage typing is used in epidemiological tracing of infections or outbreaks caused by *Staph. aureus*, *Salmonella*, *V. cholerae* and other bacteria (Fig. 58.3)

■ Enumerate the significance of phages.

Phages are important for the following reasons:

- Phage typing—is used as an epidemiological marker to establish the path of transmission of infectious agent and to identify the reservoir of infection
- Differentiation of bacterial species or genus into subtypes by phage typing
- Bacteriophages may act as carriers of genes from one bacterium to another—a process known as transduction, e.g. plasmid-mediated drug resistance in *Staph. aureus*
- Bacteriophages may confer the property of toxin production in some bacteria

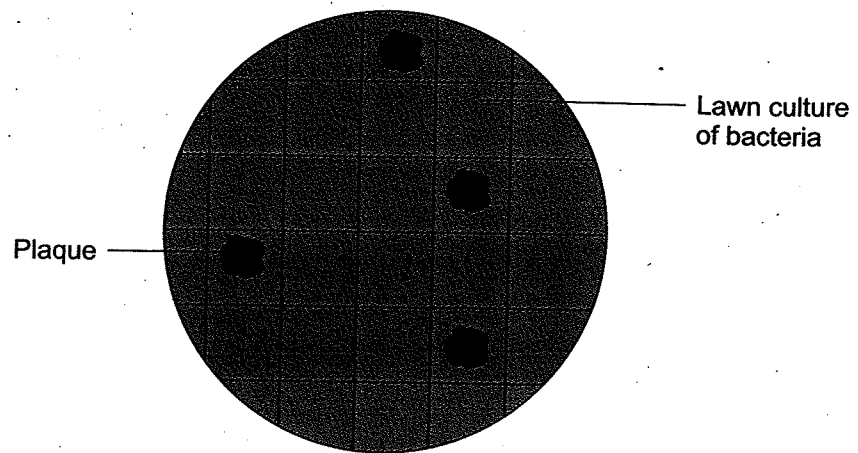


Fig. 58.3 Bacteriophage typing.

59

Chapter

Poxviruses

- List the poxviruses that cause diseases in human beings; mention their primary host and the disease caused.

Most poxviruses causing human infections belong to the genera *Orthopoxvirus* and *Parapoxvirus*, contained in the subfamily Chordopoxvirinae (see Table 59.1).

Table 59.1 Poxviruses causing human infections

Genus	Virus	Primary host	Disease
<i>Orthopoxvirus</i>	Variola	Man	Small pox
	Vaccinia	Man	Vaccine virus
	Monkey pox	Monkeys	Smallpox-like disease
	Cow pox	Cows	Localized primary vaccinia-like lesion
<i>Parapoxvirus</i>	Orf	Sheep	Contagious pustular dermatitis
	Milker's node	Cows	Small ulcerating node
Unclassified	<i>Molluscum contagiosum</i>	Humans	Benign skin lesion
	Tana pox	Monkeys	Pox-like lesion

- Mention the important properties of smallpox virus.

Properties of smallpox virus (variola virus):

- Poxviruses are largest and most complex viruses
- They can be seen under light microscope. The properties are:
 - Size: Largest virus— $300 \times 200 \times 100$ nm
 - Shape: Brick shaped
 - Genome: Double-stranded DNA—a disk shaped
 - Capsid: Double layered
 - Envelope: Lipoprotein envelope
 - Lateral body: It is a lense-shaped structure on either side of the genome
- It contains a DNA-dependent RNA polymerase (Fig. 59.1)
- The virus has only one stable serotype

- Mention the factors affecting the survival of poxviruses.

- Poxviruses can withstand drying for months—in dry state they can resist 100°C for 5–10 minutes; in moist state they are destroyed at 60°C for 10 minutes
- They can resist 50% glycerol and phenol, but are highly sensitive to formalin

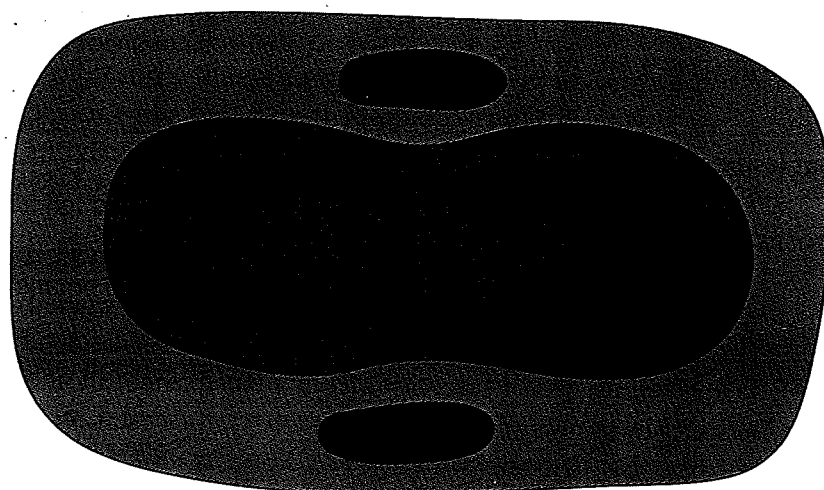


Fig. 59.1 Poxvirus.

■ **What are the methods by which poxviruses are cultivated?**

Poxviruses can be cultivated in the following ways:

- Grow on **chorioallantoic membrane** of 11–13 days old chick embryos producing small, shiny, white, convex, non-necrotic and nonhaemorrhagic pocks in 48–72 hours
- **Tissue culture**—cultivated on tissues such as monkey kidney, human carcinoma of cervix cell line (HeLa), etc.
- **Cytopathic effects**—poxvirus grows slowly and produces eosinophilic inclusion bodies called **Guarnieri bodies**. These are aggregations of virus particles in a matrix
- **Isolation**—by using animals such as monkeys

■ **Very briefly describe the antigenic structure of small poxvirus.**

- Nucleoprotein Ag is common to all members of the family
- Small poxvirus possesses a number of Ags—20 different Ags have been identified by immunodiffusion. These include LS Ag—complex of heat labile and stable Ags, agglutinin and haemagglutinin
- Variola and vaccinia are antigenically similar and differ by not more than one Ag

■ **Trace the course of disease development from the point when small poxvirus enters the host.**

The course of disease development is presented in Flowchart 59.1.

■ **Describe the clinical features of infections caused by small poxvirus.**

The following are the clinical features of small poxvirus infections:

- Incubation period: 7–14 days
- The disease is characterized by prodromal symptoms—fever and malaise followed by the rash, which first appears on the buccal mucosa (exanthem) and then spreads over the body. The rash evolves through stages from macules to papules, vesicles, pustules and scab. This course takes about 2 weeks. The scabs fall off leaving deep, pitted scars, some of which may be permanent

■ Which specimens and laboratory methods are used for diagnosing small poxviruses?

Specimens

- Scrapings from lesions
- Vesicle fluid
- Contents of pustule
- Scabs
- Blood

Methods of Diagnosis

- Demonstration of virus Ag by immunofluorescence
- Demonstration of virus by Gutstein's methyl violet or Gipsen's silver impregnation method under light microscope
- Demonstration of virus under electron microscope
- Detection of Ag by precipitation in gel or CFT
- Virus isolation on chorioallantoic membrane of chick embryo or tissue culture
- Ab detection by precipitation in gel

■ What prophylactic measures have been in use for preventing small poxvirus infections?

- Smallpox has been eradicated by global use of live attenuated vaccinia virus vaccine
- Routine vaccination is now stopped

■ Mention salient features of infections caused by the following poxviruses: (a) Cowpox and Milker's Node, (b) Monkeypox, (c) ORF, (d) Tanapox, (e) *Molluscum contagiosum* and (f) Vaccinia

(a) Cowpox and Milker's Node

- Cowpox occurs in cattle as ulcer of teats and contagious parts
- Human infection is acquired from cows as an occupational disease (transmitted during milking)
- Localized lesion develops on the hands or fingers of humans (macules to pustules) resembling primary vaccinia
- **Milker's node**—is also an occupational disease that humans acquire by milking infected cows. It is similar to cowpox (ulcerating nodules), but rarely becomes pustular

(b) Monkeypox

- It causes pox disease in monkeys
- It causes disease resembling smallpox in human beings
- Person-to-person transmission is rare

(c) ORF

- It is a contagious pustular dermatitis or sore-mouth of sheep and goats
- It is transmitted to man by contact
- It occurs as a single papulovesicular lesion with a central ulcer on hand or finger or sometimes on forearm or face

Virus enters the host by inhalation through mucosa of upper respiratory tract

↓
Multiplies in lymphoid tissue

↓
Enters the blood (primary viraemia)

↓
Reaches internal organs and multiplies

↓
Re-enters the blood (secondary viraemia)

↓
Spreads to the skin

Flowchart 59.1 Mechanism of disease development in small poxvirus infection.

(d) Tanapox

- It is the cause of a febrile illness along the Tana River in Kenya
- It forms single pock-like lesion on the upper part of the body
- The virus is antigenically unrelated to other poxviruses
- It does not grow in eggs. It is grown in human and monkey tissue cultures

(e) *Molluscum contagiosum*

- It produces benign epidermal, pink or pearly white warts on the skin of the arms, legs, buttocks and genitals
- It occurs only in humans, usually in children and young adults
- Sections of the nodules show eosinophilic hyaline inclusion bodies called **molluscum bodies** with proliferated epidermal cells and are composed of large number of virus particles, embedded in a protein matrix
- Virus cannot be grown in tissue culture, egg or animals

(f) *Vaccinia*

- It does not occur in nature.
- Cowpox virus used for vaccine production against smallpox underwent changes and became differentiable from original *Cowpox virus* known as *Vaccinia virus*, which is similar to *Variola virus* causing smallpox
- *Vaccinia virus* is studied in great detail because it is safer to work with
- It was used for small pox vaccination
- Presently, it is used as vector for incorporating genes and for developing recombinant vaccines

60

Chapter

Herpesviruses

■ Mention the general properties of the family Herpesviridae.

General Properties

- The family Herpesviridae consists of enveloped DNA viruses affecting man and animals
- It includes eight important human pathogens
- Their characteristic property is to persist indefinitely within the host cell (latent infection) and to undergo periodic reactivation producing symptoms

■ Mention the general properties of Herpesviruses.

General Properties

- All herpesviruses are structurally similar
- Size: 20–200 nm in diameter
- Capsid: Icosahedral—162 capsomeres
- Envelope: Icosahedral core is surrounded by a lipoprotein envelope
- Genome: Linear double-stranded DNA
- They replicate in the nucleus, form intranuclear inclusion bodies and obtain their envelopes from nuclear membrane
- They are heat labile and susceptible to ether, alcohol, chloroform and bile salts

■ Classify herpesviruses into subfamilies. Mention the characteristic features of each subfamily.

On the basis of biological, physical and genetic properties, herpesviruses are divided into three subfamilies (Table 60.1).

Table 60.1 Subfamilies of herpesviruses

Subfamily Sr. No.	<i>Alphaherpes virinae</i>	<i>Betaherpes virinae</i>	<i>Gammaherpes virinae</i>
1.	Replicate fast (12–18 h)	Replicate slowly (24 h)	Replicate in T or B lymphocytes
2.	Variable host range	Narrow host range	Narrow host range
3.	Latent infection in sensory ganglia	In salivary glands	In lymphoid tissue
4.	Rapidly cytopathic in culture	Grow best in fibroblasts, produce cytomegaly	—
5.	Released from cell	Remain associated with the cell	—
6.	Examples Herpes simplex virus Varicella-zoster virus	Cytomegalovirus	Epstein-Barr virus

■ Name herpesviruses of medical importance.

Eight important human pathogens in the category of herpesviruses are as follows:

1. Human herpesvirus 1—Herpes simplex virus Type 1
2. Human herpesvirus 2—Herpes simplex virus Type 2
3. Human herpesvirus 3—Varicella-zoster virus
4. Human herpesvirus 4—Epstein-Barr virus
5. Human herpesvirus 5—Cytomegalovirus
6. Human herpesvirus 6
7. Human herpesvirus 7
8. Human herpesvirus 8

~~LSN~~ Enumerate the general properties of herpes simplex virus (HSV).

General Properties

- Size: One of the largest viruses, 150–200 nm in diameter
- Capsid: Icosahedral
- Envelope: Loose, amorphous envelope derived from the nuclear membrane of the host cell
- Genome: Double-stranded DNA
- Envelope: It contains lipid, carbohydrates and protein and shows projections from its surface (Fig. 60.1)

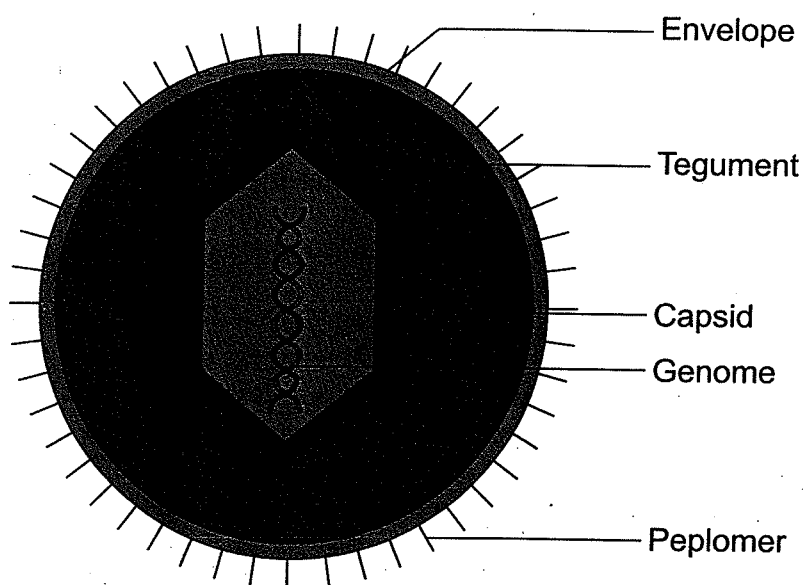


Fig. 60.1 Herpes simplex viruses.

~~LSN~~ Which animals do herpes simplex viruses infect? How can they be grown?

Animal Susceptibility

- Natural infection is seen in man only
- Experimental infection can be produced in rabbits, guinea pigs, mice, hamsters and rats

Growth

- They grow in eggs and in a variety of primary and continuous cell cultures such as
 - Monkey or rabbit kidney
 - Human amnion
 - HeLa, HEP-2 and Vero cell lines
- It produces characteristic CPE in cell cultures—formation of syncytia or giant cell formation
- On CAM of chick embryo—small (less than 0.5 mm), white, shiny, non-necrotic pocks

■ Describe the antigenic structure of herpes simplex virus.**Antigenic Structure**

- Many proteins are present, which are antigenic—some of them are nonstructural but efficient Ags
- Some Ags are common to both Type 1 and Type 2 because of antigenic sharing, they cross-react serologically and can be differentiated by using type-specific monoclonal Abs only

Differentiation of Type 1 and Type 2 (HSV)

Type 1 (HSV-1) and Type 2 (HSV-2) are distinguished on the basis of:

- **Antigenicity:** Type 1 and Type 2 are antigenically differentiated by using type-specific monoclonal Abs
- **Pock size:** Type 2 forms larger pocks on chick embryo CAM than Type 1
- **Replication in chick embryo fibroblast cells:** Type 2 replicates well, while Type 1 replicates poorly
- **Location of lesions:** In general, Type 1 causes lesions above the waist, whereas Type 2 causes lesions below the waist
- **Resistance to antiviral agents:** Type 2 is more resistant to idoxuridine (IUD) or cytarabine in cell cultures than Type 1

■ Discuss the pathogenicity of herpes simplex virus.**Pathogenicity**

- Man is the only natural host
- Saliva, skin lesions or respiratory secretions from infected cases or asymptomatic carriers (especially in genital infection) are the important sources
- HSV-1 is transmitted by direct contact or inhalation—lesions occur mainly on the face
- HSV-2 is transmitted by sexual contact causing lesions in genital area
- In persons enjoying abnormal sexual practices (oral-genital sex)—HSV-1 can cause infections of the genitals and HSV-2 can cause lesions in the oral cavity
- The virus primarily infects and replicates in epithelial cells of the skin or mucous membrane at the initial site of infection. After local multiplication cell-to-cell spread occurs and involves local lymph nodes
- The virus secondarily infects nervous tissue. From primary site of infection, it migrates up the neuron and becomes latent in the sensory ganglion cells
- HSV-1 becomes latent in trigeminal ganglia, while HSV-2 becomes latent in lumbar and sacral ganglia
- Periodic reactivation of virus from the latent state can occur, during which virus migrates down the neuron and replicates in skin and mucous membrane causing lesions
- The reactivation is induced by certain nonspecific stimuli such as sunlight, hormonal changes, trauma, emotional stress, fever, etc.

✓ Clinical Features

Both HSV-1 and HSV-2 cause primary and recurrent diseases.

Lesions by HSV-1

1. Mucosal

- Commonest site is buccal mucosa
- Acute gingivostomatitis in children is characterized by fever, irritability and vesicular lesions in the mouth, which ulcerate and become secondarily infected
- Lesions heal spontaneously in 2 or 3 weeks

2. Cutaneous

- The typical lesions are fever blisters or herpes fibrils caused by reactivation of the virus by fever
- The typical skin lesions are thin walled, umbilicated vesicles that contain serous fluid filled with virus particles and cell debris, which break down to form ulcers
- Commonly seen on the face—on cheeks, chin around the mouth and on the forehead. Also appear on the buttocks as **napkin rash** in infants
- Herpetic whitlow—occupational disease in dentists, doctors and nurses—occurs as a pustular lesion of the finger or hand
- Eczema herpeticum—generalized eruption in children suffering from eczema

3. Ophthalmic

- Acute keratoconjunctivitis characterized by corneal ulcers and lesions of conjunctival epithelium
- Follicular conjunctivitis with vesicle formation on the lids
- Recurrences can lead to scarring and blindness

4. Central Nervous System

- Aseptic meningitis and encephalitis in premature babies
- Acute necrotizing encephalomyelitis in older children and adults—associated with high mortality rate

5. Herpes Labialis (fever blisters or cold sores)

It is characterized by crops of vesicles, usually at the mucocutaneous junction of the lips or nose.

Lesions by HSV-2

1. Genital Herpes

It is characterized by vesiculoulcerative lesions on the penis and urethra in a male, and the cervix, vulva, vagina and perineum in a female. The lesions are painful and may be associated with fever, malaise and inguinal lymphadenopathy. Infection may be asymptomatic in female.

2. Neonatal Herpes

Infection may be acquired *in utero*, during birth or after birth. The infection may be:

- Localized to the skin, mouth or eyes
- Generalized—involving multiple organs, including central nervous system causing encephalitis, which is associated with high mortality and sometimes with or without localized skin involvement

Describe the laboratory methods performed for diagnosing infections caused by herpes simplex virus.

Specimens

- Vesicle fluid, skin swab, corneal scrapings, throat swab, saliva, CSF and brain biopsy.

Microscopic Examination

- Smear is prepared from the scrapings obtained from the base of vesicles and stained with toluidine blue (1%)—the multinucleated giant cells with faceted nuclei and homogeneously-stained ground glass chromatin—are seen in positive smear
- Giemsa stain—is used for demonstration of intranuclear inclusion bodies in the smear
- Electron microscopy—is used for demonstration of virus particles in smear
- Direct immunofluorescence test—is used for demonstration of virus Ag in the smears or sections from lesions
- The fluorescent Ab test using brain biopsy material—is the method of choice for rapid diagnosis of encephalitis

Virus Isolation

- It remains the definitive diagnostic approach
- Tissue culture is the method of choice
- The specimen is inoculated in tissue culture
- The appearance of typical CPE in cell culture in 2 or 3 days suggests the presence of HSV
- Identification of agent is established by using neutralization test or immunofluorescence test with specific antisera
- Further differentiation of HSV-1 and HSV-2 may be made by using specific antisera or restriction endonuclease analysis of viral DNA

Serological Tests

- These tests are useful in the diagnosis of primary infections only
- Abs develop in 4–7 days after infection and reach peak in 2–4 weeks
- Abs can be measured by
 - Neutralization test—quantitative test
 - CFT
 - ELISA
 - RIA
 - Immunofluorescence test
 - Passive haemagglutination test—rapid diagnosis of encephalitis
- Serological tests are not widely used, as diagnostic value is limited because of multiple Ags shared by HSV-1 and HSV-2. The use of type specific Abs, however, is more meaningful

Which drugs are useful in treating herpes simplex virus?

- Acyclovir—is the drug of choice for encephalitis. It is also beneficial in primary genital herpes
- Vidarabine—is also effective against neonatal herpes and encephalitis. It may be useful for the treatment of acyclovir-resistant HSV
- Idoxuridine, trifluorothymidine and vidarabine and acyclovir are also effective as topical agents for the treatment of ocular lesions

✓ 52 Describe Varicella-zoster virus (VZV) infections.

- Varicella (chickenpox) is a mild, highly contagious disease of children with a generalized vesicular eruption of skin and mucous membrane. The disease occurs following primary infection with virus
- Zoster (shingles) is a sporadic disease of adults that occurs as a result of reactivation of the latent virus present in the sensory ganglia when immunity falls to ineffective level. It is characterized by localized vesicular eruptions of skin limited to the skin supplied by the affected nerves
- Both the aforementioned diseases are caused by the same virus, HSV-3, hence named VZV

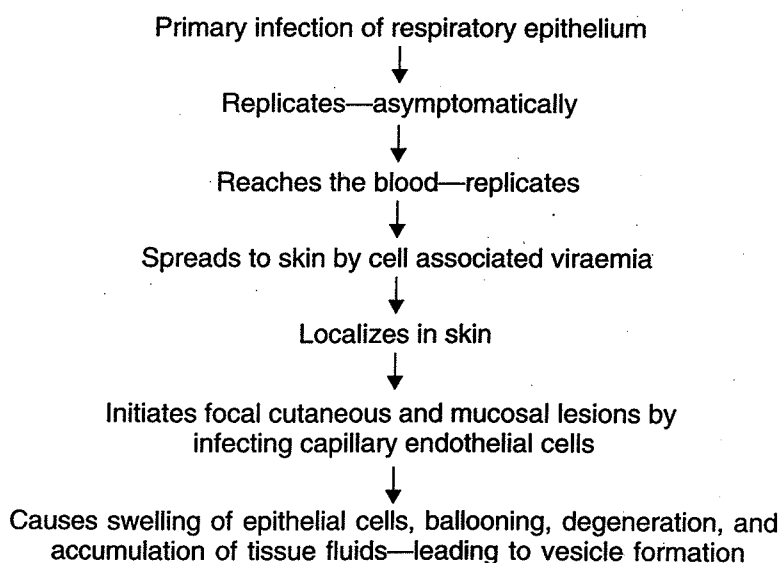
■ Mention the general properties of Varicella-zoster virus (VZV).

General Properties

- VZV is morphologically similar to HSV
- It does not grow in experimental animals and chick embryos
- It can be grown in human fibroblasts, human amnion or HeLa cells with typical intranuclear inclusion bodies
- The CPE are similar to HSV but are less marked
- Only one type of VZV is known
- By using highly specific antisera, it can be distinguished from HSV-1 and HSV-2

■ Diagrammatically represent the course of disease development in case of infection by Varicella-zoster virus.

- The virus enters through the upper respiratory tract or the conjunctiva
- During the course of infection, it infects peripheral sensory nerves where it remains in a latent phase
- Source—case of chickenpox or zoster
- The course of disease development is diagrammatically depicted in Flowchart 60.1



Flowchart 60.1 Mechanism of disease development in Varicella-zoster virus infection.

✓ Describe clinical infections produced by varicella-zoster virus.

Clinical Infections

Chickenpox (Varicella)

- Incubation period: 7–23 days
- It is highly infectious disease, characterized by
 - Malaise and fever—earliest symptoms
 - Rash—first on the trunk and then on the face, the limbs and the buccal and pharyngeal mucosa
 - The rash progresses through macule, papule, vesicle, pustule and scab
 - The rash is centripetal in distribution and superficial
 - In adults, the disease runs a more severe course than in children
 - The rash may become haemorrhagic
 - Varicella pneumonia is commonly seen, which may be fatal

Complications

- Secondary bacterial infections, usually by staphylococci and streptococci
- Reye's syndrome—acute hepatic failure, encephalopathy and hypoglycaemia
- Varicella encephalitis—may be fatal or lead to brain damage
- Varicella pneumonia in children
- The virus may cross placenta and infect the fetus during pregnancy causing congenital malformations

Herpes Zoster

- It is a disease of old age but may occur at any age in persons who had chickenpox several years earlier
- It occurs as a result of reactivation of virus in the sensory ganglia because of drop in the number of Abs or disappearance of Abs
- The virus travels along the sensory nerve and produces zoster lesions on the area of skin or mucosa
- The disease is characterized by:
 - Fever and malaise
 - Severe neuritic pain
 - Crop of vesicles over the skin supplied by the affected nerves
 - Unilateral eruption
 - Involvement of trunk, head and neck
 - Ophthalmic zoster—occasionally

Complications

- Lower motor neuron paralysis
- Generalized zoster in immunocompromised individuals with vesicles scattered throughout the skin

✓ How is the laboratory diagnosis of Varicella-zoster virus infections performed?

Specimens

Scrapings or fluid aspirated from the vesicular lesions are collected with aseptic precautions and transported immediately.

Microscopic Examination

- Demonstration of multinucleated giant cells by haematoxylin and eosin staining
- Demonstration of intranuclear inclusion bodies by Giemsa or Papanicolaou's stain

- Demonstration of intranuclear virus Ag by fluorescent Ab technique using monoclonal Abs
- Electron microscopy—to differentiate VZV from poxviruses

Virus Isolation

- Virus can be isolated in human fibroblast, human amnion or Vero cells in 3–7 days
- CPE may take more time to develop
- Isolates of VZV can be identified by immunofluorescence or other immunologic tests using specific antisera

Serological Tests

- VZV specific IgM Ab detection by ELISA
- CFT, neutralization test and indirect immunofluorescence tests are used for demonstration of specific Ab
- Counter current immunoelectrophoresis—a rapid and simple test for detection of virus Ag

How can Varicella-zoster virus infections be treated?

- Acyclovir and vidarabine are effective
- VZV immunoglobulin of high specific Ab titre prepared from pooled plasma of patients can be used to prevent the development of illness in immunocompromised children

What prophylactic measures can be adopted to prevent Varicella-zoster virus infections?

Prophylactic measures that can be adopted are, as follows:

- A live attenuated varicella vaccine (Oka strain) induces good Ab response and provides protection for several years (given subcutaneously). This vaccine was very labile
- A modified lyophilized form of the vaccine is now available, which is safe and effective. It is recommended for children in the age group of 1–12 years as single dose and for older individuals two doses (6–10 weeks apart). It is to be administered subcutaneously

Enumerate the general properties of cytomegalovirus (CMV).

General Properties

- CMV is morphologically similar to HSV and VZV
- It is the largest virus in the Herpesvirus family—150–200 nm
- Its DNA content is significantly larger than HSV
- It exhibits strict host specificity. Human CMV replicates in humans only and unable to grow/replicate in animals
- Human CMV can be grown in human fibroblast cultures. It grows slowly and requires a longer period of incubation to produce CPE
- Characteristic CPE includes—perinuclear cytoplasmic inclusions in addition to the intranuclear inclusions and multinucleated cells. Many affected cells are greatly enlarged
- The assembly occurs in the nucleus and envelope is derived from the inner nuclear membrane

Describe the pathogenesis of cytomegalovirus infections.

Pathogenesis

- CMV replicates in epithelial cells of respiratory tract, salivary glands, kidney and cervix, and is shed in urine, saliva, cervical secretions, tears, semen and breast milk
- It may be transmitted from person-to-person by close contact
- Transmission may occur by
 - Transplacental transfer—in fetus
 - Exposure to maternal body fluids during delivery and maternal breast feeding—in infants

- Contact with saliva or urine of infected individuals—in younger children
- Sexual intercourse, kissing, blood transfusion—in adults
- Infection may also be transmitted by organ transplantation
- The virus after gaining entry into body remains associated with lymphocytes and polymorphonuclear cells and may cause cell-associated viraemia

✓ ■ Write a short note on clinical features of cytomegalovirus infections.

Clinical Features

Most of the infections are asymptomatic—lead to prolonged latency with occasional reactivation.

Congenital Infections

- It may remain asymptomatic at birth or may lead to cytomegalic inclusion disease, which is often fatal
- It is characterized by hepatosplenomegaly, jaundice, thrombocytopenic purpura, haemolytic anaemia, microcephaly, permanent mental abnormalities and chorioretinitis

Postnatal Infections

- These are usually inapparent but may sometimes lead to insidious hepatitis or pneumonitis
- Primary infection in older children and adults is usually inapparent but may lead to infectious mononucleosis-like syndrome characterized by malaise, myalgia, protracted fever, liver function abnormalities and lymphocytosis without heterophile Abs (cytomegalovirus mononucleosis). The patient shows cytomegalouria, rise of CMV Abs and presence of virus in the peripheral blood leucocytes
- In immunocompromised hosts—in patients with malignancy, immunodeficiency, AIDS and transplant recipients, CMV causes more severe infections. Pneumonia, hepatitis or generalized disease are caused by reactivation of latent infection or by exogenous infection. It often causes disseminated disease in AIDS patients

✓ ■ Describe the laboratory methods used for diagnosing cytomegalovirus infections.

Specimens

Urine, saliva, breast milk, cervical secretions, semen and blood leucocytes.

Microscopic Examination

- Demonstration of cytomegalic cells—enlarged cells with large intranuclear inclusions (Owl's eye) in centrifuged deposits of urine or saliva
- Demonstration of virus by electron microscopy
- Molecular technique—DNA hybridisation

Isolation of Virus

- In human fibroblast culture—most reliable
- CPE becomes visible after 1–2 weeks
- Identification can be confirmed by staining culture cells with immunofluorescence or immunoperoxidase technique using monoclonal Abs

Serological Tests

Abs may be detected by

- Neutralisation test
- CFT

- RIA
- Immunofluorescence test
- ELISA to detect specific IgM Ab

■ Which drugs are used for treating cytomegalovirus infections?

The following drugs are used for treating cytomegalovirus infections:

1. Ganciclovir—drug of choice
2. Acyclovir—is also beneficial

✓ ■ Mention the general properties of Epstein-Barr virus (EBV).

General Properties

- EBV named after the discoverers—Epstein and Barr—who isolated this virus from Burkitt's lymphoma
- It is structurally and morphologically similar to other herpesviruses but is antigenically different. Also lacks nucleic acid homology
- It grows well in human blood lymphocytes—human B lymphocytes have receptors (CR2 or D21) for EBV, hence it specifically affects these cells
- It causes transformation of B lymphocytes, which undergo continuous multiplication. The transformed cells contain many EBV genomes

■ How is Epstein-Barr virus transmitted and which tissue is affected?

- EBV is transmitted by infected saliva
- It initially replicates in the epithelial cells of the oropharynx and parotid glands. Subsequently, B lymphocytes are infected, where it persists in latent state

✓ ■ Describe the clinical features of infections caused by Epstein-Barr virus.

Clinical Features

- EBV is ubiquitous in all human populations
- By the age of 3 years, about 80% children in developed countries and 99% children in developing countries acquire infection
- Once infected, the virus is present in an individual for life
- In most cases, it remains silent
- The following clinical manifestations may result:

1. Infectious Mononucleosis (glandular fever)

- It is an acute and self-limited disease, which lasts for 2–4 weeks
- It is characterized by
 - Pyrexia
 - Sore throat
 - Lymphadenopathy
 - Splenomegaly
 - Anorexia and lethargy
 - Hepatitis—subclinical
 - Encephalitis—in some patients
 - Presence of abnormal lymphocytes in peripheral blood
 - Common in young adults
- Entry of virus—through the respiratory route by close contact with patients
- Kissing is believed to be the common mode of transmission, hence known as **kissing disease**—most prevalent among the adolescents and young adults

2. Burkitt's Lymphoma

- Seen in children of 5–8 years age
- It affects sites such as jaw, orbital cavities and gastrointestinal tract
- It occurs as endemic type or African type—associated with EBV infection and the sporadic type in which only 20% cases show EBV infections

3. Nasopharyngeal Carcinoma

It is cancer of epithelial cells—common in males of Chinese origin.

4. Lymphomas

It occurs in immunodeficient hosts and transplant recipients.

5. Oral Hairy Leucoplakia

It is wart-like growth on the tongue, seen in HIV-infected patients and transplant recipients.

LSM Describe the laboratory diagnostic methods for identifying Epstein-Barr virus infections.

Specimens

- Saliva, peripheral blood or lymphoid tissue

Isolation of Virus

Isolation of virus is extremely difficult, time consuming (6–8 weeks) and requires special facilities; hence most EBV infections are diagnosed serologically.

Serological Tests

Two types:

1. Test for detection of EBV-specific Abs

- Commonly used serological tests are:
 - Indirect immunofluorescence test
 - ELISA
 - CFT
 - Gel diffusion
- As specific Abs (IgG) persist for long periods, its presence along with absence of IgM indicates—past infection
- Current infection is indicated by rise in Ab titre to one of the EBV Ags or by demonstrating IgM type of Abs to viral capsid Ag
- Detection of IgA Abs to EBV capsid Ag in serum appears to be useful screening test for early detection of nasopharyngeal carcinoma

2. Test for detection of heterophile Abs

- During the acute phase of infectious mononucleosis, most patients (85–90%) develop IgM Abs, which are the heterophile Abs. These Abs agglutinate sheep erythrocytes
- The heterophile Abs are detected by in which inactivated serum (56°C for 30 minutes) in doubling dilutions is mixed with equal volumes of 1% sheep RBCs suspension and incubated at 37°C for 4 hours. The tubes are observed for agglutination and a titre for 100 or above is considered suggestive of infectious mononucleosis
- As similar type of heterophile Abs may be present in the blood as a result of injection of therapeutic horse serum and even in normal individuals. The results of Paul-Bunnell test

should be confirmed by using agglutinin absorption tests. To confirm these Abs, absorption of agglutinins with Guinea pig kidney and Ox RBCs is necessary

- **Nucleic acid hybridisation:** This is most sensitive for detection of EB virus

■ **How can Epstein-Barr virus infections be treated and prevented ?**

- Antiviral therapy is not necessary in uncomplicated cases of infectious mononucleosis
- Acyclovir has little activity. High doses may be useful in life-threatening EBV infections

Prevention

No EBV vaccine is available.

61

Chapter

Adenoviruses

■ Why are adenoviruses so named?

They are named so because they were first isolated from adenoids (Rowe et al. 1953).

■ State the general properties of adenovirus.

General Properties

- Size: 70–90 nm in diameter
- Capsid: Icosahedron capsid made up of 252 capsomeres. 240 of the 252 capsomeres are hexons (have six neighbours) while 12 are pentons (have five neighbours). Each penton unit—consists of a penton base anchored in the capsid and a rod-like projection with knob attached at the distal end (known as fibre). Because of which virus appears like a **space vehicle** (Fig. 61.1)
- Genome: Double-stranded DNA associated with histone-like polypeptide
- Envelope: Absent

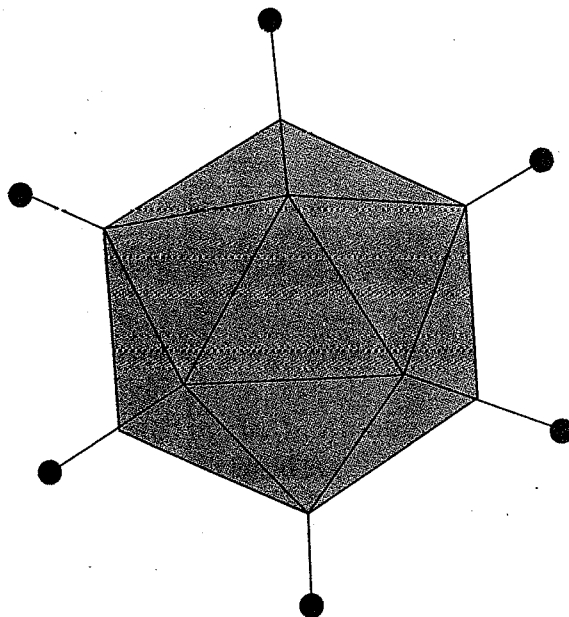


Fig. 61.1 Adenovirus.

■ Comment on cultivation of adenoviruses

- Laboratory animals are not susceptible to human adenoviruses
- Human adenoviruses can be cultured in tissue cultures of human origin, e.g. human amnion, HeLa or HEP-2 cells
- CPE—include—cell-rounding and aggregation into grape-like clusters—takes several days
- Intranuclear inclusion bodies may be seen in stained preparations

■ Mention the resistance of adenoviruses to external conditions.

Adenoviruses are relatively more stable than other viruses—remain viable for a week at 37°C. Readily inactivated at 50°C. They resist ether and bile salts.

■ Classify adenoviruses.

- Human adenoviruses are divided into six subgenera, A–F, based on their haemagglutination property, G+C content of DNA and oncogenicity.

■ Write the antigenic structure of adenovirus.

- All adenoviruses have a common group-specific Ag located on the hexon protein detected by immunofluorescence and ELISA
- Type-specific Ag is located in the polypeptides of the fibre protein detected by neutralization test
- Forty-seven antigenic types are known

■ Describe the pathogenesis of adenovirus infections.

- Entry of adenovirus occurs by droplet (aerosol) inhalation, direct inoculation of conjunctiva or faeco – oral route
- After entry in the host, the virus with the help of terminal knob-like structure (fibre) attaches to mucosal epithelial cells of respiratory tract (both upper and lower), the gastrointestinal tract or conjunctiva and enter inside the cell
- It causes death of the cell in acute infection or it may cause a latent infection, particularly in the adenoid and tonsillar tissue of the throat

■ Name the diseases caused by adenoviruses.

Diseases caused by adenoviruses are described below:

Infections of Upper Respiratory Tract

- Acute febrile pharyngitis in infants and children
- Pharyngoconjunctival fever in children
- Acute respiratory disease in young adults, characterized by fever, sore throat, coryza and conjunctivitis

Infections of Lower Respiratory Tract

- Atypical pneumonia—characterized by fever, cough, and patchy consolidation
- Usually occurs as a complication of acute respiratory disease—in children and infants

Ocular Infections

Epidemic keratoconjunctivitis and acute follicular conjunctivitis

Other Infections

- Acute haemorrhagic cystitis in children
- Gastroenteritis with nonbloody diarrhoea in children
- Generalized exanthem

■ What methods are employed for diagnosing adenovirus infections?

The commonly used methods are as follows:

1. **Direct immunofluorescence:** Virus is demonstrated in cells aspirated from the nasopharynx by direct immunofluorescence stain using group-specific Abs

2. **Culture:** Virus is isolated from the throat, conjunctiva, urine or stool by using cell cultures (HeLa, HEP-2 or KB). Growth is identified by characteristic CPE or by CFT using adenovirus antiserum
3. **Haemagglutination test:** This is performed using monkey and rat RBCs to identify the group
4. **Serological tests:** Demonstration of a four - fold or greater rise in Ab titre
CFT, Haemagglutination inhibition, neutralization and RIA tests are used.

■ **Mention the methods of treatment and prevention of adenovirus infections.**

Treatment

No antiviral therapy.

Prevention

- * No vaccine is available for general use.
- * A live non-attenuated vaccine (Types 4 and 7) is used in the form of enteric coated capsule in military recruits. Given orally—liberates virus in the intestines—produces subclinical infection and confers protection against respiratory diseases.

62

Chapter

Picornaviruses

■ Mention the important features of picornaviruses.

Important Features

- These are RNA viruses
- They are found in intestine and excreted in faeces
- Man is the only natural host
- They spread by faeco-oral route
- Size: Small, 27–30 nm in size
- Envelope: Absent
- Genome: Single-stranded RNA
- Frequently seen in children than adults and common in summer

■ List the medically important enteroviruses.

Following are the enteroviruses of medical importance:

- Polioviruses—1–3
- Cocksackie viruses—A 1–24 except 23, B 1–6
- ECHO viruses—1–34 except 10, 28
- Enteroviruses—68–71

■ Mention the morphological characteristics of poliovirus.

Morphological Characteristics

- Spherical particle—27 nm in diameter
- Icosahedral in symmetry
- Nonenveloped RNA virus—contains single strand of positive sense RNA

■ Which external conditions are favourable for the growth of poliovirus and under which conditions can it be destroyed?

- In faeces it can survive for months at 4°C and for years at –20°C
- It is resistant to bile, proteolytic enzymes and detergents
- It is inactivated by heat at 55°C for 30 minutes
- Chlorination destroys virus

■ Enumerate the antigenic properties of poliovirus.

On the basis of neutralization test viruses are classified into three types—1, 2, 3 and on the basis of CFT, ELISA and precipitation there are 2 types of Ags—C and D.

C Ag

- It is heated or H Ag
- It is associated with empty virion

- It is less specific
- Anti-C Ab is not protective

D Ag

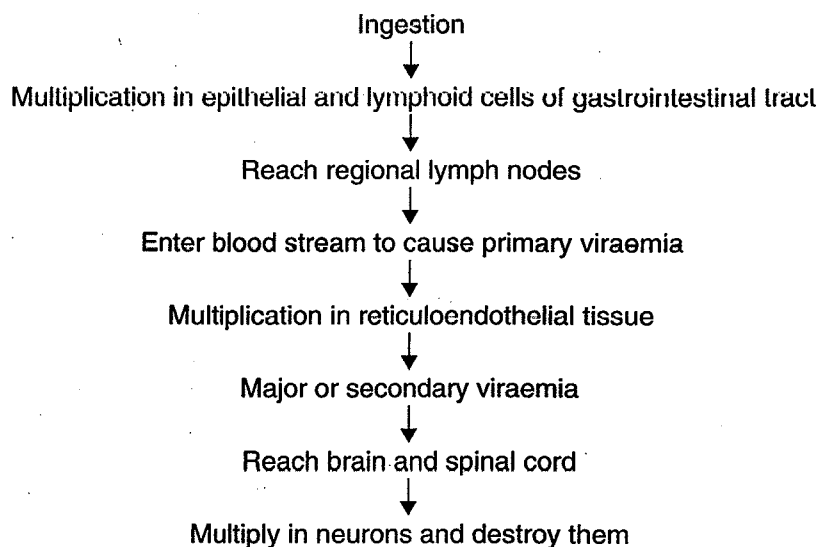
- It is native or N Ag
- It is associated with whole virion
- It is type specific
- Anti-D Ab is protective in nature
- D can be converted into C by heating at 50°C
- Potency of injectable polio vaccine is measured in terms of D Ag units

■ Comment on cultivation of poliovirus.

- Poliovirus grows in tissue cultures of simian and human origin, e.g. primary monkey kidney culture
- In culture it produces cytopathic effects in 2 or 3 days
- Infected cells round up and become refractile, pyknotic and show eosinophilic intranuclear inclusion bodies

■ Make an outline sketch to describe the pathogenesis of poliovirus.

Pathogenesis is diagrammatically depicted in Flowchart 62.1.



Flowchart 62.1 Mechanism of disease development in poliovirus infection.

■ Mention the clinical features of poliovirus infection.

Poliovirus can cause four types of infection. These are as follows:

1. Inapparent Infection

90–95% individuals develop only inapparent infection during which patient is asymptomatic but virus can be isolated from samples of stool or throat.

2. Minor Illness

Fever, sore throat, headache, malaise is associated with primary viraemia, it may progress to paralytic form.

3. Major Illness

If infection progresses, minor illness is followed by major illness characterized by headache, neck stiffness, back pain, other features of aseptic meningitis, which mark the stage of viral invasion of CNS. It is also called nonparalytic poliomyelitis.

4. Paralytic Poliomyelitis

- In some patients flaccid paralysis occurs after a stage of meningitis
- Depending on distribution, infection is bulbar, spinal or bulbospinal
- Recovery of paralysed muscles takes place in next 4–8 weeks and completes after 6 months, leaving behind varying degrees of residual paralysis

■ Describe the laboratory diagnosis of poliovirus infection.

Specimens

- Blood, CSF, throat swab, faeces
- Virus can be isolated from blood during the stage of viraemia, from throat swab in early stage of disease and from faeces in 80% of patients during the first week of infection

Direct Demonstration

This is achieved by electron microscopy and immune electron microscopy.

Isolation

- It grows in tissue cultures of human or simian origin such as primary monkey kidney, HEP-2 cells
- Growth is indicated by cytopathic effects appearing in 2 or 3 days
- Infected cells round up and become refractile, pyknotic and show eosinophilic intranuclear inclusion bodies

Identification

For identification, neutralization test is performed.

Serology

Paired sera are tested by neutralization test (Abs appear early and last lifelong) and CFT, Anti-D Abs appear after some weeks and last for 5 years.

■ Discuss prophylaxis of poliovirus infection.

Poliovirus infection can be prevented by

1. **Passive immunization:** Human Ig is of little value.
2. **Active immunization:** This is achieved by vaccination. Two vaccines are available:
 - Oral or Sabin—Sabin attenuated strain is used for preparation of vaccine
 - Injectable or Salk—It is prepared by growing viruses in tissue culture and inactivating them by formalin

Distinguishing features of Sabin and Salk strains are presented in Table 62.1.

OPV (Oral Polio Vaccine)

- It is given orally along with DPT. Three doses are sufficient to induce satisfactory immune response in developed countries, but in India more doses (up to five) are required as seroconversion is poor because of
 - Poor potency of vaccine and improper storage

Table 62.1 Differences between Sabin and Salk vaccine

	<i>Sabin</i>	<i>Salk</i>
Route of administration	Oral	Injectable
Type of vaccine	Live attenuated vaccine	Killed
Schedule of vaccination	<ul style="list-style-type: none"> • 3 doses 4–6 weeks apart • 1st booster 6 months later 	3 doses at 4–6 weeks apart Boosters every 3–5 years
Time of 1st dose	Can be given early (maternal Ab have less effect on Abs)	After 6 months (because immune response is impaired by maternal intestinal infection)
Safety	Reversion to virulent form is possible and not safe in immunodeficients	Reversion of virulence is not seen and not harmful in immunodeficients
Economy	More economical	Less economical than oral
Immunity	Systemic as well as local immunity in intestine	Systemic immunity no intestinal immunity
Induction of herd immunity	Yes	No
Duration of immunity	Long lasting	Immunity needs to be maintained by boosters

- Interference by other enteroviruses in gut
- Neutralization by antibodies in breast milk (immediate breast feeding after vaccination)
- Type of immunity—local intestinal immunity—antibody mediated

■ Mention the general features of Coxsackie virus.

General Features

- Coxsackie virus was first isolated from the village of Coxsackie in New York, therefore it is called Coxsackie virus
- It is a member of the family Picornaviridae
- Morphologically it is similar to other picornaviruses
- It is an inhabitant of alimentary canal
- It is characterized by its ability to infect suckling mice
- The characteristic feature of the virus is its ability to infect suckling mice. Adult mice are not susceptible

■ Classify Coxsackie viruses.

On the basis of pathological changes in suckling mice, viruses have been classified into the following two groups:

1. Coxsackie A
2. Coxsackie B

By neutralization test, Group A is classified into 24 types and Group B into six types. Coxsackie A-23 is same as ECHO 9 and Coxsackie A-24 is same as ECHO 34.

■ How can Coxsackie virus be cultured. Mention the cultural characters of Group A and Group B viruses?

Coxsackie virus can be cultured in the following ways:

1. By Using Suckling Mice

Following intracerebral and intraperitoneal inoculation, viruses produce the following changes in the suckling mice:

Group A—produces generalized myositis and flaccid paralysis leading to death within a week.

Group B—produces patchy focal myositis, spastic paralysis, necrosis of brown fat and pancreatitis, hepatitis, myocarditis, encephalitis.

2. By Monkey Kidney Tissue Culture

Group B grows well in it while Group A except 7 and 9, cannot grow in it.

■ List the clinical features of Cocksackie virus infection.

- Herpangina-vesicular pharyngitis
- Aseptic meningitis, encephalitis
- Fever with rash
- Minor respiratory tract infection like common cold
- Epidemic pleurodynia or Bornholm disease
- Myocarditis and pericarditis in newborn
- Juvenile diabetes
- Orchitis
- Transplacental and neonatal transmission resulting in a serious disseminated disease in newborns
- Post-viral fatigue syndrome

■ By which method is Cocksackie virus detected in a laboratory?

For detection of Cocksackie virus, isolation is by using suckling mice inoculation and identification by studying histopathological findings.

■ State the general characters of enteric cytopathic human orphan (ECHO) viruses.

- ECHO viruses are members of the family picornaviridae
- They infect enteric canal of humans
- They resemble other picornaviruses in morphology
- They are called ECHO viruses because they are unrelated to any particular diseases
- They are not pathogenic to laboratory animals.

■ How many serotypes of ECHO virus have been identified?

By neutralization test ECHO viruses have been classified into 34 serotypes. (Type 10 is reclassified as Reo-1 and Type 28 is reclassified as Rhinovirus.)

■ Comment on cultivation of ECHO viruses.

- Monkey kidney tissue culture is used and growth is detected by cytopathic effects
- The virus is not pathogenic to laboratory animals

■ What are the infections caused by ECHO viruses?

Most of the ECHO viruses cause asymptomatic infection. They can cause:

- Common cold
- Aseptic meningitis

- Encephalitis, ascending paralysis
- Morbilliform rash
- Neonatal infection
- Gastroenteritis

■ **How is ECHO virus infection diagnosed in a laboratory?**

Faeces, throat swab, and CSF are collected by standard procedures and inoculated in cultures. Study of cytopathic effect helps in identification. Neutralization test may also help in identifying serotypes.

■ **Which viruses are included in the group 'other enteroviruses'? Name the diseases caused by each one of them.**

- Newly discovered enteroviruses are included in this group
- They are enteroviruses 68 to 72 associated with human diseases

Pathogenicity

- Enterovirus 68—causes pneumonia
- Enterovirus 70—causes acute haemorrhagic conjunctivitis
- Enterovirus 71—causes meningitis
- Enterovirus 72 is hepatitis A virus—causes hepatitis

■ **State the salient features of rhinovirus.**

- Rhinovirus belongs to the family picornaviridae
- It causes common cold
- Morphologically resembles other picornaviruses
- Destroyed at acid pH 3–5
- Pathogenesis: Grows in nasal mucosa and causes upper respiratory tract infections
- Clinical features: Coryza with fever in adults and children
- Complications: Sinusitis, bronchitis and otitis media
- Laboratory diagnosis: Nasopharyngeal swab collection, culture on human tissue cultures and detection by study of cytopathic effects help in laboratory diagnosis

63

Chapter

Orthomyxoviruses

■ Mention the general properties of myxoviruses.

General Properties

- Myxoviruses are spherical or filamentous RNA viruses
- They have the characteristic ability to adsorb on mucoprotein receptors of erythrocytes that lead to haemagglutination
- They are divided into following two families:
 1. Orthomyxoviridae
 2. Paramyxoviridae

■ Enumerate the differences between orthomyxoviruses and paramyxoviruses.

Features that differentiate ortho- from paramyxoviruses are presented in Table 63.1.

Table 63.1 Differences between orthomyxoviruses and paramyxoviruses

<i>Orthomyxoviruses</i>	<i>Paramyxoviruses</i>
1. 80–120 nm in size	100–300 nm in size
2. Spherical or filamentous	Pleomorphic
3. Genomic RNA is segmented and have 8 pieces	Genome is a single linear RNA
4. Sensitive to RNase	Resistant to RNase
5. Nucleocapsid is 9 nm in diameter	Nucleocapsid is 18 nm in diameter
6. Antigenically variable	Antigenically stable
7. Genetic reassortment—common	Genetic reassortment—absent
8. Actinomycin D inhibits multiplication	Actinomycin D does not inhibit
9. Haemolysin is absent	Haemolysin is present
10. Causes influenza	Causes parainfluenza, respiratory syncytial disease, mumps and measles

■ Mention the morphological characteristics of influenza virus.

Morphological Characteristics

- Influenza virus is spherical in shape or may be pleomorphic
- Size: about 80–100 nm in diameter
- Filamentous forms are also seen
- Nucleocapsid has helical symmetry and consists of eight segmented RNA and RNA- dependent RNA polymerase
- Nucleocapsid is surrounded by a protein layer, which in turn is surrounded by lipid bilayer envelope
- Two spikes project from surface—these are haemagglutinin and neuraminidase

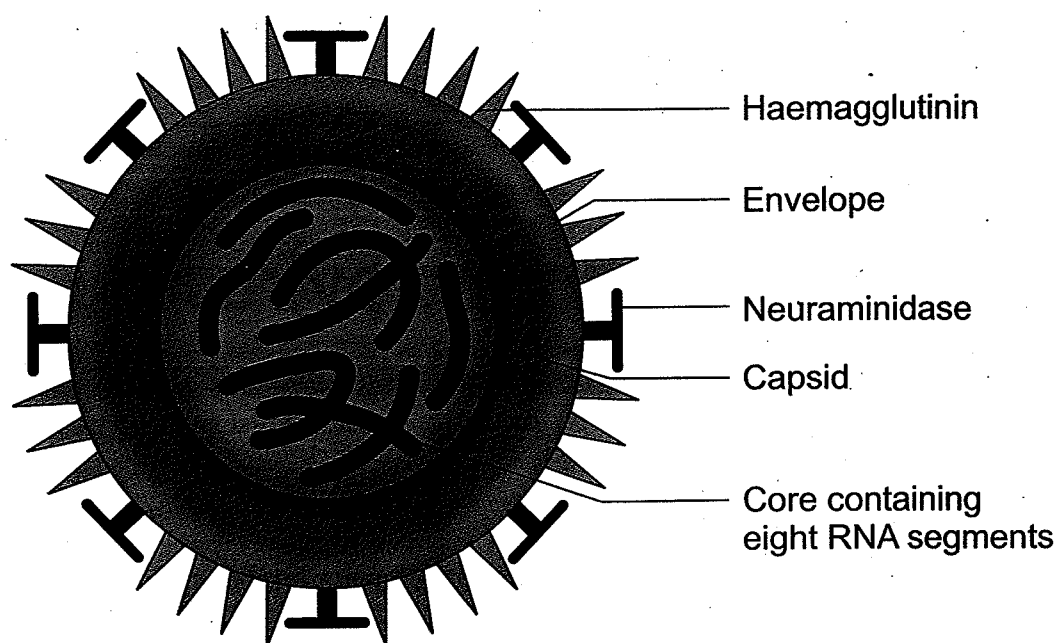


Fig. 63.1 Influenza virus.

- Haemagglutinin (H) spikes or peplomers are tapering, triangular in cross-section 10 nm in length and are 50/virion
- Neuraminidase (N) peplomers or spikes are mushroom-shaped projections 9 nm in length and 100/virion (Fig. 63.1)

✓ **Discuss the process of haemagglutination in influenza virus. How is it helpful in detection of the virus?**

- Haemagglutination is a characteristic feature of influenza virus
- When mixed with erythrocytes of fowl, virus gets adsorbed on surface and thus links adjacent cells to cause haemagglutination
- Haemagglutinin peplomer is responsible for causing haemagglutination
- Haemagglutination is followed by a detachment of virus from cell, which is called elution; it is brought out by the enzyme neuraminidase present in neuraminidase peplomer
- Eluted virus particles from red cells are still capable of agglutinating fresh RBCs, but red cells that have been acted on by the virus are not susceptible to agglutination by same strains of the virus. This is due to the destruction of specific receptors
- Neuraminidase acts on the cell receptor and destroys it, which leads to reversal of haemagglutination
- Haemagglutination can occur at 0°–37°C and viruses vary in their ability to cause haemagglutination
- Types A and B can agglutinate fowl, human and guinea pig RBCs while C agglutinates fowl RBC at 4°C only

Uses

1. Haemagglutination inhibition is a useful method for detection and quantitation of Abs produced against it
2. Haemagglutination and elution can be used for purification and concentration of influenza viruses
3. Haemadsorption—cells in which virus is multiplying contain haemagglutinin, therefore red cells get adsorbed on the surface of such cells, this technique is used to detect viral growth in cultures

4. Haemagglutination can be used for the titration of killed influenza virus, e.g. standardization of killed influenza virus vaccine

■ **To which external factors does influenza virus offer resistance?**

- Influenza virus is inactivated by heat
- It remains viable on fomites for 2 weeks
- It is susceptible to many disinfectants such as formaldehyde, phenol and iodine

■ **Describe the structure of antigens present in influenza virus.**

Antigenic structure comprises the following:

Ribonucleoprotein (RNP) Ag

- It is type-specific Ag and based on it influenza viruses are classified into three types—A, B and C
- It is stable and does not undergo variation
- It can be demonstrated by complement fixation and immunoprecipitation tests

Haemagglutinin (H)

- It is a glycoprotein, which is strain-specific
- It undergoes variation
- Abs to it are produced following the infection and immunization
- Abs are protective, act by preventing adsorption of virus to cell
- It is responsible for haemagglutination and haemadsorption
- It has 15 distinct subtypes, H1 to H15

Neuraminidase (N)

- It is a glycoprotein, which destroys cell receptors and brings out elution
- It is strain-specific and also exhibits variations
- Abs to it are formed after infection and immunization
- Abs are not effective in protection like Abs of haemagglutinin.
- Abs inhibit release and spread of progeny virion and thus limit infection
- It has 9 subtypes (N1–N9)

✓ **Describe the antigenic variations in influenza virus.**

- Antigenic variability is highest in Type A, less in Type B, while not demonstrated in Type C
- RNP Ag is stable, but H and N undergo antigenic variations
- Variation are of two types:
 1. Antigenic drift
 2. Antigenic shift

≠ **Antigenic Drift**

- It is a gradual, sequential change in antigenic structure occurring at frequent intervals
- Newer Ags are related with previous Ags, so they react with antisera to previous viruses
- It occurs due to **mutation** in H and N Ags
- It is responsible for periodic epidemics of influenza

≠ **Antigenic Shift**

- It refers to abrupt, drastic, discontinuous variation in antigenic structure
- New viral Ags are unrelated with previous viral Ags so Abs to previous virus cannot neutralize them
- It is due to **genetic recombination**
- It is responsible for major epidemics and pandemics

■ Describe the system of nomenclature in use for influenza virus.

- System of nomenclature includes—influenza virus Type A/B, place of isolation, strain number and year of first isolation followed by the antigenic subtypes (H and N subtypes), e.g. A/Hong Kong/1/68 (H3N2)
- Influenza A has subtypes, they share common RNPAg but differ in H and N Ags

■ Describe the methods by which influenza virus can be cultivated and detected?

Cultivation and subsequently detection of influenza virus can be achieved by growing the virus on the following media:

Chick Embryo

- Viruses can be grown in amniotic cavity of 1–3-day old chick embryo
- Influenza A and B grow well in amniotic cavity and after few egg passages, in allantoic cavity but Type C can be grown in amniotic cavity only
- Viral growth is detected by appearance of haemagglutinin in amniotic and allantoic fluids

Tissue Cultures

- Primary monkey kidney and some continuous cell lines are used
- Growth is detected by haemadsorption or demonstration of haemagglutinin titre in culture fluid or by immunofluorescence

■ Discuss the pathogenesis and clinical features of influenza virus infection.

Pathogenesis and Clinical Features

- Virus enters through the respiratory tract
- Neuraminidase facilitates infection by hydrolyzing the mucus in respiratory tract and exposing cell receptors for viral adsorption
- Death of infected cell occurs and this renders respiratory tract vulnerable to secondary bacterial infection
- The disease is characterized by sudden onset of fever with chills, nonproductive cough, myalgia and headache

Complications

- Croup
- Pneumonia
- Middle ear infection
- Secondary bacterial infections
- Reye's syndrome
- Myocarditis
- Encephalitis

■ Describe the laboratory diagnosis of influenza virus infection.

Specimens

Nasopharyngeal secretions, nasal swab, throat garglings.

Collection

Specimens are collected in buffered salt solution. If cannot be processed immediately, can be stored at 4°C.

Demonstration of Virus Ag

- It can be demonstrated by immunofluorescence
- Detection of viral nucleic acid—This is accomplished by reverse transcriptase—polymerase chain reaction (RT-PCR)

Isolation

- **Embryonated eggs**
 - Sample is inoculated in amniotic cavity and after an incubation period of 3 days at 35°C, eggs are harvested and fluid tested for haemagglutination titre
- **Tissue culture**
 - Monkey kidney cell cultures are used and growth is detected by haemadsorption and immunofluorescence
 - Isolate can be confirmed by complement fixation test and haemagglutination inhibition

Ab Detection

Paired sera are tested to demonstrate rise in titre by CFT and Haemagglutination test.

■ **Which drugs can be recommended for treating influenza virus infection?**

Drugs that can be recommended for treating influenza virus infection are amantadine and zanamivir.

■ **Write the prophylactic measures for influenza.**

The following prophylactic measures can prevent influenza:

- **Killed vaccine:** Virus grown in allantoic cavity is inactivated by formalin or beta-propiolactone and purified
- **Subunit vaccine:** Killed vaccine contain haemagglutinin and neuraminidase subunits
- **Recombinant vaccine**
- **Live attenuated vaccine—Ts (temperature sensitive) mutant vaccine:**
 - Vaccine strain grows at lower temperature of nasopharyngeal mucosa at 32°–34°C but not in lungs at 37°C
 - It can be administered by aerosols or intranasally
 - It stimulates local IgA production

■ **What is swine flu?**

- Swine influenza or “Swine Flu” or “Hog Flu” or “H1N1 Influenza” is a highly contagious acute respiratory disease caused by any strain of the influenza virus endemic in pigs (swine) that regularly causes outbreaks of the influenza among pigs
- Strains endemic in swine are called swine influenza virus (SIV)

■ **Describe the swine flu virus and comment on its origin.**

- Morphologically, it is similar to influenza virus but antigenically different
- An apparent reassortment of at least four strains of influenza A virus, subtype H1N1, including one strain endemic in humans, one endemic in birds and two endemic in swine resulted in formation of novel virus. This is called quadruple reassortment of virus
- Present swine flu virus, the 2009 flu outbreak is due to a new strain of influenza virus

■ **Describe the role of pigs in development of new swine flu virus.**

Pigs cells possess receptors that can bind to swine, human and avian influenza viruses; therefore, two or more types of viruses may co-infect swine cells and combine to produce a novel virus.

When influenza viruses from different species infect pigs, the viruses can reassort and new viruses that are mix of swine, human and/or avian influenza viruses can emerge

■ Describe the modes of transmission of swine flu.

- Spreads from person to person
- Transmitted like seasonal flu
- Passed to other people by exposure of infected droplets expelled by coughing or sneezing
- Infected droplets can also contaminate hands or surfaces, such as the door handles, computer keyboards, mobiles, ordinary phones and TV remote control

152 ■ Describe the laboratory diagnosis of swine flu.

Specimens

- Nasal swab, nasal wash, nasopharyngeal aspirate, nasopharyngeal swab, sputum, broncho-alveolar lavage, transtracheal aspirate, etc.

Collection

- Samples should be collected by hospital staff (not by laboratory staff) by observing safety measures
- More than one specimen should be collected before antiviral medication
- Swab with a synthetic tip polyester or Dacron with aluminium or plastic shaft should be used. Dry swab should be inserted in nostrils back to nasopharynx and left in place for 10 seconds and slowly removed while rotating it slightly and placed in viral transport medium and labelled

Transport

- Store specimen at 4°C before and during transportation
- It should reach within 48 hours to the laboratory
- Store specimen at -70°C, if time required is beyond 48 hours
- Do not store in freezer and avoid freeze-thaw cycles
- Transport in a viral transport medium in appropriate packaging
- Send sample with consent form and clinical data sheet to NIV Pune/NICD, Delhi, maintaining cold chain

Specimen Processing

Specimens should be processed in a class 3 safety cabinet (glove box maintained under negative pressure with HEPA filtered air and double HEPA filtered exhaust).

Confirmatory test

- **Real time (RT)-PCR**
 - It is a quantitative PCR, which detects viral RNA in less than one hour
 - It is diagnostic, however, does not indicate stage of disease
- **Viral culture**
 - Cell cultures are used for cultivation
 - Detects viral growth in 2-10 days
- **Limitations of culture**
 - Sample should be collected within three days of illness
 - Negative culture does not rule out infection
 - Results depend on the quality of specimen and conditions of the storage/transport
 - May not give timely results for clinical management of patient

- **Neutralisation**
 - Four-fold rise in virus-specific Abs in paired sera
- **Genomic sequencing**

Rapid tests

- **Rapid influenza diagnostic tests (RIDTs)**
 - BinaxNOW® Influenza A and B
 - Directigen™ EZ Flu A+B and QuickVue influenza A+B
- Immunofluorescence

Advantages of the rapid tests

- Rapid -15–30 minutes
- Good results: when viral load is high
- Detect or distinguish A and B

Limitations of the rapid tests

- Negative tests do not rule out infection
- Fail to detect virus when viral load is low
- Detects virus group-specific antigen and not swine flu Ag
- Low sensitivity: 42–69%, which is affected by quality, collection time, storage and transport conditions of the specimen
- False positive can occur

■ Write about treatment and prevention of swine flu.

- **Prevention**
 - Social distancing
 - Respiratory etiquette
 - Hand hygiene
 - Personal protective equipments
 - Avoid spitting
 - Avoid direct contact with hands
 - Vaccine
 - Chemoprophylaxis
- **Treatment**
 - Tamiflu (Oseltamivir)
 - Relenza (Zanamivir)

64

Chapter

Paramyxoviruses

■ Name the family and genera to which paramyxoviruses belong. List important pathogens belonging to these genera.

Family: Paramyxoviridae

Genus: Paramyxovirus

– Mumps virus

– Parainfluenza virus

Genus: Morbillivirus

– Measles virus

Genus: Pneumovirus

– Respiratory syncytial virus

■ Enumerate the general properties of paramyxoviruses.

- Morphologically they resemble orthomyxoviruses but are larger and more pleomorphic
- Size: 100–300 nm, sometimes up to 800 nm
- Shape: Roughly spherical
- Genome: One piece of single-stranded RNA
- Nucleocapsid: Helical
- Envelope: Lipoprotein envelope with two types of transmembrane spikes—(a) Haemagglutination (H) and (b) Fusion protein (F) (Fig. 64.1)

■ Mention the pathogens belonging to the genus *Paramyxovirus*. Also mention their hosts.

The genus *Paramyxovirus* includes the following viruses:

- The mumps virus—infects human beings
- The parainfluenza virus—infects human beings and other mammals

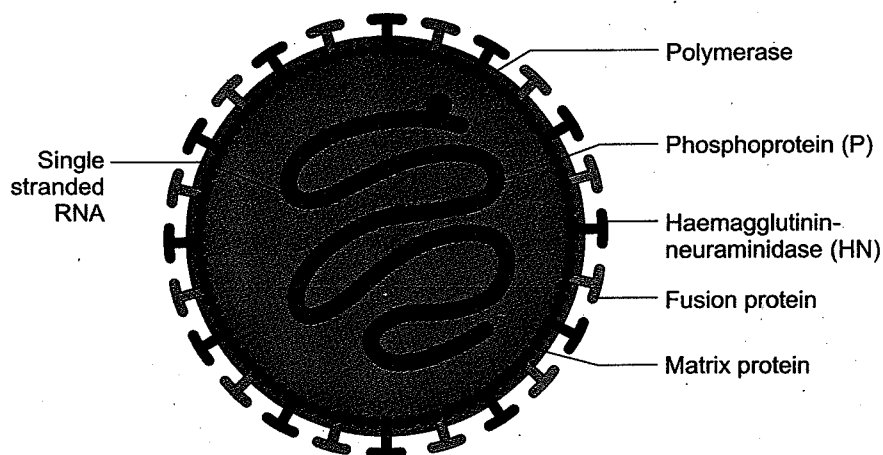


Fig. 64.1 Paramyxovirus.

- The avian paramyxoviruses—include the Newcastle disease virus of chickens and many other species infecting birds

✓ Mention the morphological characteristics of mumps virus.

Morphological Characteristics

- It is a spherical virus
- Size: 100–250 nm in diameter
- Genome: Single-stranded RNA
- Envelope: It is composed of a lipoprotein membrane and is covered by projections:
 - **Haemagglutinin (H)**—protein responsible for adsorption of virus to the host cell. It may have neuraminidase activity, hence also known as HN
 - **Fusion protein (F)**—responsible for fusion of viral envelope with a plasma membrane of host cell. It also mediates haemolysis

■ Write down the methods of cultivation for mumps virus.

- Mumps virus grows in chick embryo in amniotic cavity (primary isolation) and in allantoic cavity after adaptation, incubated at 35°C for 5 days
- It also grows in primary monkey kidney cell culture, human amnion or HeLa cells—cytopathic effects (CPE) produced are syncytium formation and acidophilic cytoplasmic inclusions. CPE take more time to manifest, hence for detection of growth haemadsorption and immunofluorescence are better option

■ Mention the antigenic structure of mumps virus.

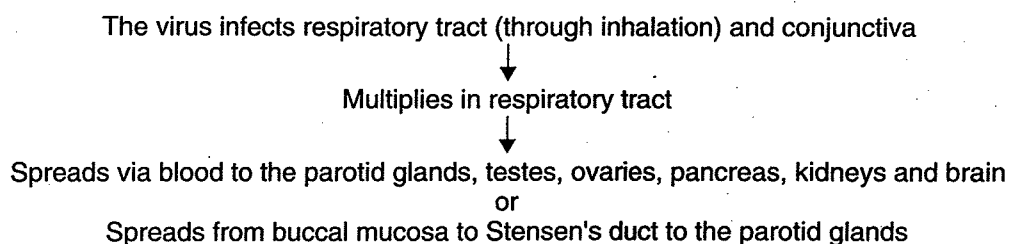
- Antigenically mumps virus is stable. Only one serotype is known
- There are two complement fixing antigens:
 1. The soluble Ag (S)—the internal nucleocapsid Ag
 2. The viral (V) antigen

■ How can mumps virus be inactivated?

Mumps virus can be rapidly inactivated at room temperature by ultraviolet rays and by disinfectants like formaldehyde and ether.

■ Diagrammatically represent how mumps infection develops in the body.

Development of mumps or epidemic parotitis is depicted in Flowchart 64.1.



Flowchart 64.1 Pathogenesis of mumps.

■ Describe the clinical features of mumps.

Clinical Features

- Mumps is a disease of childhood characterized by nonsuppurative enlargement of parotid glands
- Incubation period: 12–25 days
- The disease is characterized by a prodromal stage of fever, malaise and anorexia followed by tender swelling of the parotid glands—either unilateral or bilateral
- It is typically benign and resolves spontaneously within a week

Complications

Important complications include:

- Orchitis in post-pubertal males, which if bilateral, can result in sterility or low sperm count. If unilateral—it is quite painful but does not lead to sterility
- Meningitis—benign, self-limited and without any sequelae
- Other complications such as pancreatitis, oophoritis and nephritis may occur rarely

■ Describe the laboratory diagnostic procedures of mumps.

Most cases are diagnosed clinically and laboratory support is not required generally. The following tests are available:

Microscopy

Demonstration of virus in saliva or throat secretions by immunofluorescence test.

Culture

- The virus can be isolated in cell cultures from saliva, spinal fluid, or urine. The specimen is inoculated on primary monkey kidney cell culture, human amnion or HeLa cells. Growth takes 1 or 2 weeks. Growth is detected by haemadsorption and identified by haemadsorption inhibition test using specific antiserum. CPE take more time
- The virus can also be isolated in chick embryo by amniotic route and testing amniotic fluid after 5 or 6 days for haemagglutination. The virus can be identified by using haemagglutination inhibition test. Egg inoculation is a less sensitive method

Serological Tests

- Haemagglutination test or complement fixation test—a four-fold increase in antibody titre is diagnostic
- Complement fixation test that assays both S and V Ags can also be used—Abs to S indicate current infection (as Abs to S appear early and are short-lived). Abs to V indicate past infection only
- For rapid diagnosis—ELISA for detection of mumps specific IgM antibodies

■ How is mumps infection treated and prevented?

Treatment

No specific antiviral therapy is available.

Prevention

- Mumps infection gives life-long immunity
- A live attenuated vaccine is effective and gives long lasting immunity (at least 10 years)
- It can be administered subcutaneously at the age of 15 months, usually in combination with attenuated measles and rubella vaccine as MMR vaccine

■ Write a note on parainfluenza virus.

General Properties

- Morphologically similar to other paramyxoviruses
- Surface spikes—constituted of haemagglutinin (H), neuraminidase (N) and fusion protein (F). H and N are present on same spike
- Both humans and animals are infected
- Four types are known based on antigenicity, cytopathic effects and pathogenicity

Pathogenicity

- Infection via respiratory route—by inhalation
- Cause upper and lower respiratory tract disease without viraemia
- Large number of cases are of subclinical infection
- Parainfluenza Types 1 and 2 are major cause of croup—the most serious clinical disease. Also cause pharyngitis
- Type 3 causes bronchitis, bronchiolitis and pneumonia
- Type 4—minor respiratory disease

Clinical Features

- It is the main cause of
 - **Croup**—acute laryngotracheobronchitis in children under 5 years—characterized by harsh cough and hoarseness
 - Other respiratory disease, e.g.
 - Common cold
 - Pharyngitis
 - Bronchitis
 - Pneumonia

Laboratory Diagnosis

Specimens

Throat swab, nasal swab.

Isolation

- Primary monkey kidney cell cultures or continuous monkey kidney cell lines are used
- CPE are not readily apparent, hence haemadsorption test is used to detect growth

Serological Tests

- Fourfold or greater rise in Ab titre is diagnostic
- ELISA, neutralization test and CFT are helpful in diagnosis

■ Write a short note on Newcastle disease virus (NDV).

- NDV is a single-stranded RNA virus
- It is a natural pathogen of poultry. In India it is known as **Ranikhet virus**
- Transmission occurs by exposure to faecal and other excretions from infected birds, and through contact with contaminated feed, water, equipment and clothing
- In humans it causes self-limited conjunctivitis in poultry workers and others in contact with birds
- Vaccination and slaughtering of infected birds are the control measures

■ Mention the characteristic morphological features of measles virus.

Morphological Features

- Like other paramyxoviruses, it is a spherical virus and is pleomorphic
- Size: 120–250 nm in diameter
- It possesses a lipoprotein envelope having haemagglutinin (H) spikes but no neuraminidase activity
- It also has fusion protein (F) that mediates cell fusion and haemolytic activities

■ Write the methods of cultivation and detection of growth for measles virus.

Cultivation

- This virus does not grow in chick embryo
- Human or monkey kidney cell culture and human amnion culture are used for primary isolation
- It can be adapted for growth on continuous cell lines (HeLa, Vero) and in the amniotic sac of hen's egg

Detection

- Cytopathic effects, studied for its detection are—multinucleate syncytium formation with acidophilic nuclear and cytoplasmic inclusions
- Multinucleated giant cells (Warthin–Finkeldey cells) are also found in the lymphoid tissue of patients

■ Which external factors can inactivate measles virus? How can it be made to resist heating uptill 50°C for 1 hour?

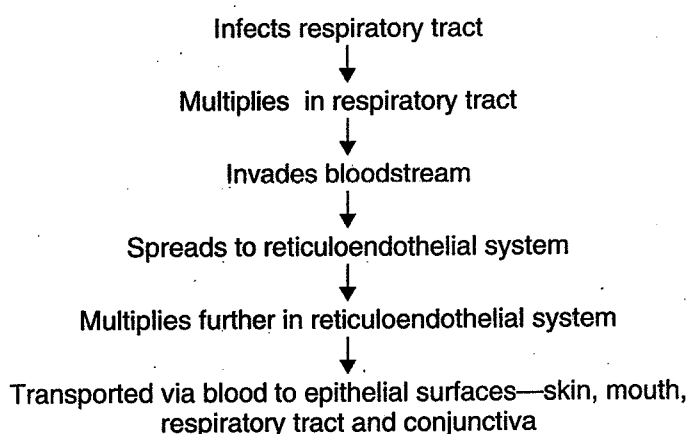
- It is a labile virus, readily inactivated by heat, ultraviolet light, ether and formaldehyde
- It can be stabilized by molar MgSO_4 , so that it resists heating at 50°C for 1 hour

✓ ■ Explain the antigenic structure of measles virus.

The lipoprotein envelope of measles virus having haemagglutinin (H) is antigenic. The neuraminidase Ag is absent. It also has fusion (F) protein. It is antigenically uniform. It shares antigens with the viruses of canine distemper and bovine rinderpest.

✓ SN ■ Describe the pathogenicity of measles virus.

- Measles is also known as **rubeola**
- It is a highly infectious childhood disease transmitted by inhalation of virus present in respiratory secretions of patients
- The spread of infection in the body is depicted in Flowchart 64.2



Flowchart 64.2 Mechanism of disease development in measles virus infection.

✓ ■ Describe the clinical features of measles.

Clinical Features

- Incubation period: 10–14 days
- Prodromal stage characterized by fever, conjunctivitis, malaise, running nose and cough

- **Koplik's spots**—bright red lesions with a white, central dot on buccal mucosa are diagnostic
- A few days later—a red maculopapular rash appears first on the face and spreads downwards. Rash disappears in the same sequence after 3–6 days leaving behind a brownish hue

Complications

- Most patients recover from the disease
- A few develop following complications due to virus:
 - Croup
 - Bronchitis
 - Fatal giant cell pneumonia
 - Subacute sclerosing panencephalitis (SSPE)—a rare and late complication. It is a fatal disease of the central nervous system
 - Protracted diarrhoea in children from poor nations
 - In pregnant women—spontaneous abortion or premature delivery
 - Thrombocytopenia leading to purpura and bleeding from the mouth, intestine and genitourinary tract
- Complications due to secondary bacterial infections are:
 - Pneumonia
 - Otitis media—quite common

✓ Describe the ways in which measles can be diagnosed in a laboratory.

- Most cases of measles are diagnosed clinically
- In atypical cases or to differentiate it from rubella—laboratory tests are useful. The following methods are used:

Microscopy

Demonstration of multinucleated giant cells in Giemsa-stained smears of nasal secretions for early diagnosis, even before the rash appears.

Direct immunofluorescence test

Demonstration of virus in nasal secretions by direct immunofluorescence test.

Culture

Isolation of virus from nose, throat, conjunctiva and blood during prodromal phase and up to 2 days after the rash appears and from urine for a few more days using primary human or monkey kidney cell culture or human amnion. As CPE takes longer time, early detection of growth is done by immunofluorescence test

Serological Tests

- ELISA for detection of IgM in single specimen
- Haemagglutination inhibition, complement fixation and neutralization tests can also be used for detection of antibodies—demonstration of fourfold rise using sera collected during the acute phase and 10–21 days later
- Demonstration of high titre of Abs in CSF is used for diagnosis of SSPE

■ Mention the ways of treatment and prevention of measles infection.

Treatment

No specific antiviral therapy is available.

Prevention

- Normal human immunoglobulin—within 6 days can prevent or modify the disease. It is valuable in children with immunodeficiency, pregnant women and others at special risks
- A safe and effective live attenuated vaccine is given subcutaneously in children at the age of nine months—gives protection for 20 years. Booster dose is not necessary.
- This vaccine can be given in combination with mumps and rubella as MMR
- A live attenuated vaccine given by intranasal aerosol in young babies offers good protection

■ State the general properties of respiratory syncytial virus.

- Respiratory syncytial virus is morphologically similar to other paramyxoviruses
- It is pleomorphic, 150–300 nm in size
- The envelope has two surface spikes—glycoproteins, *viz.*
 - The G protein by which it attaches to cell surfaces
 - The fusion protein—causes cell to fuse, forming syncytia, which gives rise to the name of the virus
- It does not possess haemagglutinin (H), neuraminidase (N) and haemolytic properties

■ By which methods can respiratory syncytial virus be cultured?

- Respiratory syncytial virus does not grow in eggs
- Cultivation can be done in heteroploid human cell cultures such as HeLa and Hep-2

■ How can respiratory syncytial virus be inactivated and preserved?

- Respiratory syncytial virus is highly labile, inactivated rapidly at room temperature
- It can be preserved by lyophilization

■ How many types of antigens are known in respiratory syncytial virus?

- Respiratory syncytial virus is antigenically stable and only one antigenic type is known
- Studies using monoclonal antibodies have identified two subtypes—A and B

■ Name the natural hosts of respiratory syncytial virus.

Humans and chimpanzees are the natural hosts of respiratory syncytial virus.

■ Describe the pathogenicity of respiratory syncytial virus.

- Infection occurs by inhalation or direct contact of contaminated hands with nose or mouth. Infection is localized to respiratory tract and no viraemia occurs
- RSV causes outbreak of respiratory infections every winter
- Infection is more severe in infants, often involving lower respiratory tract than in older children and adults, in whom it causes mild upper respiratory tract infections

■ What are the clinical features of infections caused by respiratory syncytial virus?

Clinical Features

- In infants
 - Lower respiratory tract disease
 - Tracheobronchitis
 - Bronchiolitis
 - Pneumonia
 - Secondary bacterial pneumonia—rare

- In older children and adults:
 - Upper respiratory tract infections resemble the common cold
 - Otitis media in young children
 - Pneumonia may occur

■ How can respiratory syncytial virus infections be diagnosed in a laboratory?

Specimens

Nasopharyngeal swab or nasal washings.

Processing of Specimen

Microscopy

Rapid detection of virus by immunofluorescence on smears of respiratory epithelium.

Culture

- In HeLa or HEP—two cell cultures—immediate inoculation of specimen after collection is necessary
- Observed for CPE—characteristic giant cell and syncytium formation—takes longer time (10 days)
- Early detection of growth by immunofluorescence test

Serological Diagnosis

By demonstration of rising Ab titre of at least fourfold by ELISA, CFT, neutralization test or indirect immunofluorescence test.

■ Suggest methods of treating and preventing respiratory syncytial virus infections.

Treatment

Ribavirin administration by continuous aerosol in severely ill, hospitalised infants.

Prevention

- No effective vaccine is available
- Nosocomial outbreak can be limited by hand washing and use of gloves

65

Chapter

Arboviruses

■ What are arboviruses?

- Arboviruses are a group of viruses that are biologically transmitted by bite of haematophagous insect vectors (usually mosquitoes and ticks) in which they multiply
- Ability to multiply in arthropods and transmission are their special characteristic
- There are about 500 arboviruses that cause infections in vertebrates
- More than 100 can infect humans
- In India over 40 arboviruses have been detected, out of that more than 10 viruses are known to produce human diseases.

■ Name the viruses belonging to each of the five taxonomic families of arboviruses.

Arboviruses belonging to the five taxonomic families are as follows:

- | | |
|---------------------------------|--|
| Family: | Togaviridae |
| Examples of Arboviruses: | <ul style="list-style-type: none">– Chikungunya virus– O'nyong'nyong virus– Mayaro virus– Semliki forest virus– Sindbis virus– Ross river virus– Eastern equine encephalitis virus– Western equine encephalitis virus– Venezuelan equine encephalitis virus |
| Family: | Flaviviridae |
| Examples of Arboviruses: | <ul style="list-style-type: none">– Japanese encephalitis virus– West Nile virus– St. Louis encephalitis virus– Ilheus virus– Yellow fever virus– Dengue fever virus– Kyasanur forest disease virus– Omsk haemorrhagic fever virus– Murray valley encephalitis virus– Louping ill virus |

Family: Bunyaviridae

Examples of Arboviruses:

- California encephalitis virus
- Sandfly fever viruses
- Nairobi sheep disease virus
- Ganjam virus
- Hantan virus
- Reoviridae
- Colorado tick fever virus
- Crimean-Congo haemorrhagic fever viruses

Family: Rhabdoviridae

Examples of Arboviruses:

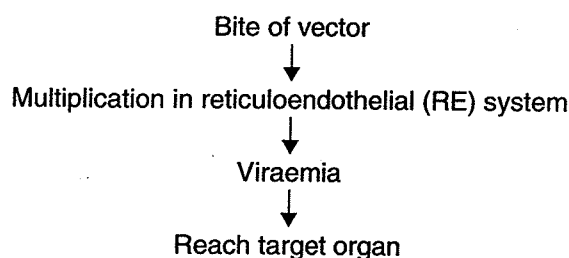
- Vesicular stomatitis virus
- Chandipura virus

■ **Mention the characters that are common to arboviruses.**

- Arboviruses are all RNA viruses, 40–100 nm in size and spherical in shape
- They multiply in arthropods, usually ticks and mosquitoes
- They are transmitted by bite of arthropods in which they multiply
- Mice is commonly used for isolation of viruses (It is the most sensitive method for isolation) though they can grow in many tissue cultures and chick embryo
- They agglutinate RBCs of goose and chicks
- They possess haemagglutinins, complement fixing and neutralizing Ags
- They are susceptible to heat, bile salts, ether and other lipid solvents

■ **Briefly describe the pathogenesis of arboviruses infection.**

Arboviruses infections are zoonotic. Arthropods get infected by biting to viraemic vertebrates and after extrinsic incubation period they become infective (Flowchart 65.1).



Flowchart 65.1 Pathogenesis of arboviruses infection.

■ **Mention the clinical features of arboviruses infections.**

Clinical Features

- Fever with or without rash and arthralgia
- Encephalitis
- Haemorrhagic fever
- Characteristic systemic disease of target organ

Viruses Causing Fever With or Without Rash and Arthralgia

- Chikungunya virus
- O'nyong'nyong virus
- Mayaro virus
- Sindbis virus
- West Nile virus
- Ross River virus
- Dengue fever virus

These viruses are transmitted by mosquitoes.

- Colorado tick fever virus
- Sandfly fever viruses

These viruses are transmitted by ticks.

Viruses Causing Encephalitis

- Eastern equine encephalitis virus
- Western equine encephalitis virus
- Venezuelan equine encephalitis virus
- Japanese encephalitis virus
- West Nile virus
- St. Louis encephalitis virus
- Murray valley encephalitis virus
- California encephalitis virus
- Louping ill virus

All are transmitted by mosquito except Louping ill virus, which is transmitted by ticks.

Viruses Causing Haemorrhagic Fever

- Chikungunya virus
- Yellow fever virus
- Dengue fever virus
- Kyasanur forest disease virus
- Omsk haemorrhagic fever virus
- Crimean Congo haemorrhagic fever viruses

■ Which tests are performed in a laboratory for diagnosing arbovirus infections?**Specimens**

- Blood, CSF, brain tissue

Culture

- Animal inoculation—suckling mice is inoculated by intracerebral route
- Tissue culture—chick embryo fibroblast
 - Vervet monkey kidney cell line (VERO)
 - HeLa cells
- Embryonated hen's egg—yolk sac
- Isolate is identified by haemagglutination and immunofluorescence

Serology

Ab detection by:

- Haemagglutination inhibition test
- CFT

- Gel precipitation
- Immunofluorescence test
- ELISA
- Neutralization test

■ **Name the arboviruses that are prevalent in India. Describe the aetiology and clinical features of the infections caused by each virus. Add a note on prevention and control.**

Arboviruses Prevalent in India

- Chikungunya virus
- Japanese encephalitis virus
- Sindbis virus
- Kyasanur forest disease virus
- Sandfly fever virus
- Chandipura virus
- Ganjam virus
- Dengue fever virus
- West Nile virus

Aetiology and Clinical Features, Prevention and Control

Chikungunya virus

It is a virus causing febrile illness with arthralgia

- **Vector:** *Aedes aegypti*
- No animal reservoir identified.
- **Geographical distribution:** Africa, India (Maharashtra), South East Asia
- **Clinical features:** Severe joint pain is the characteristic feature
 - Fever, severe joint pain, lymphadenopathy, conjunctivitis and maculopapular rash are other symptoms
- **Prevention and control:** control of mosquitoes

Japanese encephalitis virus

- Disease was first recognized in Japan, therefore called Japanese encephalitis
- **Vector:** *Culex tritaeniorhynchus*. Vector has predilection to cattle than pigs and humans and the high cattle ratio in India limits its spread to humans
- **Geographical distribution:** Asia
- Major national health problem in India—Tamil Nadu, Assam, Uttar Pradesh, Haryana, west Maharashtra and Goa
- **Clinical features:**
 - Majority of infections are asymptomatic
 - Acute onset high continuous fever, headache, vomiting
 - Signs of encephalitis, convulsions, coma
- **Prevention and control:**
 - Vaccine—2 doses at interval of 2 weeks and booster after 6–12 months
 - Control of mosquitoes
 - Establishment of piggeries away from residential area

Kyasanur forest disease virus

- **Vector:** Tick—transmission occurs by bite of ticks
- **Geographical distribution:** Karnataka, India

- **Clinical features:** Acute onset fever, headache, myalgia, vomiting, haemorrhages in skin, mucosa and viscera
- **Prevention and control:** Control of ticks and vaccination with killed KFD vaccine

Sandfly fever virus

- **Vector:** Sandfly—Transmission occurs by bite of sandfly (*Phlebotomus papatasi*)
- **Geographical distribution:** Northwest India, Mediterranean, tropical America
- **Clinical features:** Three days fever, self-limiting, nonfatal
- **Prevention and control:** Control of sandfly and protection against the bite of sandfly

Chandipura virus

- Multiplies in sandfly
- **Geographical distribution:** Isolated from blood of patient suffering from chikungunya fever from Nagpur (MS)
- Pathogenicity is not yet established

Ganjam virus

- **Vector:** Transmitted by ticks
- **Geographical distribution:** Orissa, India
- **Clinical features:** Mild febrile illness
- **Prevention and control:** Control of ticks

LSN.

Dengue fever virus

- Four types of dengue viruses—1 to 4
- **Vector:** Mosquito—*Aedes aegypti*
- **Morphology:** It is a Flavivirus, 40–100 nm size, spherical in shape.
- **Geographical distribution:** Tropical and subtropical countries
- **Pathogenesis**
 - Arthropod *Aedes aegypti* gets infected by biting to viraemic vertebrates
 - After extrinsic incubation period it becomes infective, when bites human beings releases viruses, which multiply in RE (reticuloendothelial) system causing viraemia and transfer of viruses to kidney, liver, spleen and bone marrow
- **Clinical features:** Incubation period is 2–7 days. It causes classical dengue or dengue haemorrhagic fever
- **Classical dengue**
 - Older children and adults
 - Acute onset fever with severe muscle pain, breakbone fever, headache, retrobulbar pain, conjunctival inflammation, pain in back and limbs, flushing of face
 - Erythematous maculopapular rash
 - Mild haemorrhagic manifestations—epistaxis, petechiae, purpura
 - Lymphadenopathy
- **Dengue haemorrhagic fever**
 - With haemorrhagic manifestations
 - Thrombocytopenia, haemoconcentration
 - Pleural effusion, ascites, hypoproteinaemia
 - Hypotension leading to dengue shock syndrome
 - DIC
 - Renal failure may occur

- **Laboratory diagnosis**

Specimens

Whole blood, serum, plasma, CSF, tissue biopsy, postmortem tissue.

- A. **During early stage of disease (before day 5 of illness)**
 - a. **Virus isolation** – using tissue culture—chicken embryo fibroblast, Vero and HeLa. Mosquito cell lines C6/36 or AP-61 can also be used. Growth is detected by cytopathic effects or immunofluorescence.
 - b. **Ag detection** – NS1 (Nonstructural protein) Ag or E/M (Envelope and membrane) Ag - detection by IgM ELISA
 - c. **Nucleic acid detection** - RNA detection by PCR and real time PCR.
- B. **At the end of acute phase**
 - a. **Ab detection** - IgM detection by Dot ELISA and Dipstick (Rapid tests), and IgM Ab capture ELISA (MAC ELISA)
Seroconversion from negative to positive IgM Ab to dengue indicates recent infection
 - b. **IgG** – ELISA – four-fold rise in titre in acute and convalescent sera indicates acute infection
 - c. **IgM / IgG ratio** - used to distinguish primary from secondary dengue virus infection. It is greater than 1:2 in primary and less than 1:2 in secondary dengue.
 - d. **Haemagglutination inhibition test** - during secondary dengue titre exceeds 1:1280
 - e. **PRNT (plaque reduction and neutralization Test)** - measures titre of neutralizing Ab in infected person
- **Prevention and control:**
 - Control of mosquitoes
 - No effective vaccine

West Nile virus

- It is a flavivirus
- **Vector:** Culex mosquito
- **Geographical distribution:** Africa, Central Asia, India, Mediterranean area
- **Clinical features:** It causes dengue-like illness
- **Prevention and control:** Control of mosquitoes

66

Chapter

Rhabdoviruses

■ What are rhabdoviruses? Mention their family and genera?

Rhabdoviruses are bullet-shaped, enveloped, RNA viruses.

Family: Rhabdoviridae

Genera: Vesiculovirus—Vesicular stomatitis virus

Lyssa virus—Rabies virus

SN: ■ Enumerate the morphological characteristics of rabies virus.

Morphological Characteristics (Fig. 66.1)

- Rabies virus is bullet-shaped with one end rounded or conical and other planar or concave
- Size: 180 nm × 75 nm
- It is an enveloped virus; lipoprotein envelope carries knob-like spikes composed of glycoprotein G
- Spikes do not cover the planar end of virion
- Beneath the envelope is a membrane or matrix M protein layer, which may be invaginated at the planar end. Membrane projects from planar end of some virion forming a bleb
- Core of virion consists of helically arranged ribonucleoprotein, which is unsegmented, linear single-stranded RNA
- Nucleocapsid also contains RNA-dependent RNA polymerase



Fig. 66.1 Rabies virus.

■ Mention the external factors to which rabies virus is sensitive and factors, which can inactivate it.

- Rabies virus is sensitive to alcohol, iodine preparations, soap, detergents and lipid solvents such as ether, acetone
- It is inactivated by phenol, formalin, beta-propiolactone, sunlight and ultraviolet rays
- It can survive for weeks when stabilized by 50% glycerol at room temperature

■ How can rabies virus be cultured?

Rabies virus can be grown in

1. Animals
2. Chick embryo
3. Tissue culture

Animals

- Mice is the animal of choice. After inoculation by intracerebral route, they develop encephalitis and die within 5–30 days
- Virus isolated from natural case is termed as **street virus** and after serial intracerebral passages in rabbit virus becomes fixed
- Differences between street virus and fixed virus are summarized in Table 66.1

Table 66.1 Differences between street and fixed virus

<i>Street virus</i>	<i>Fixed virus</i>
Isolated from natural case of rabies	After several serial intracerebral passages in rabbit street virus undergoes change and becomes fixed
Following inoculation by any route it can cause fatal encephalitis in laboratory animals	More neurotrophic, less infective by other routes to experimental animals
Fatal encephalitis is developed after long variable incubation period 1–12 weeks	Fatal encephalitis is produced after short and fixed incubation period 6–7 days
Negri bodies can be demonstrated in animal dying of street virus infection	Negri bodies are not usually demonstrable in animal dying of fixed virus infection

Chick Embryo

Yolk sac is used for inoculation (duck egg can also be used)

Tissue Culture

It can grow in primary and continuous cell lines such as chick embryo fibroblast but cytopathic effects are not apparent and yield is low. Fixed virus can be grown in human diploid cells, Vero cell cultures and chick embryo

■ Describe the antigenic structure of rabies virus.

The following constitute the antigenic structure of rabies virus:

1. Surface Spike-glycoprotein G

- It is important in pathogenesis, virulence and immunity
- It mediates binding of virus to acetylcholine receptors in neural tissue
- It induces haemagglutination inhibition (HI) and neutralizing Abs and stimulates cytotoxic T cells
- It is a serotype-specific Ag
- It may provide safe and effective vaccine
- HI Abs develop following the infection and immunization and parallel neutralizing Abs
- HI test helps in determining immunity to rabies but it is less sensitive and some inhibitor in sera limit their value

2. Nucleocapsid Protein

- It is group-specific Ag that induces complement fixing Abs
- It is not protective
- Antisera prepared against it are used in diagnostic immunofluorescence test

3. Other Ags

- Membrane protein
- Glycolipid
- Nucleic acid-dependent RNA polymerase

LSN

■ Which disease is caused by rabies virus? Draw an outline sketch of the path of disease development.

- Rabies virus is the causative agent of **rabies**
- Infection in humans is called hydrophobia because person exhibits fear of water, being incapable of drinking though subjected to intolerable thirst. This feature is not seen in animals
- The course of disease development is depicted in Flowchart 66.1

Bite of rabid dog (rarely through licks, aerosols and transplantation of cornea or other infected tissue)



Virus present in saliva of animal deposited in the wound



Multiplies locally in connective tissue, muscles and in nerves



Penetrates nerve endings



Travels in axoplasm towards spinal cord and brain
(speed in axons 3 mm/hour)



Spreads centripetally from axons to neuronal bodies and progressively up to spinal cord



Reaches brain and multiplies



Spreads centrifugally via nerve trunks



Infects various parts of body including salivary glands



Multiplies in salivary glands



Shed in saliva, also in milk and urine.
Virus is also present in cornea and facial skin

Flowchart 66.1 Development of rabies.

Describe the clinical features of rabies.

Clinical Features

- Incubation period is short when wound is on the face and hands and long when on legs
- It is shorter in children than adults
- Incubation period is related with period required by virus to reach the brain
- Four stages are seen in humans:
 1. Prodromal phase
 2. Acute neurological phase
 3. Coma
 4. Death

Prodromal Phase

It shows the following signs/symptoms:

- Fever, headache, malaise, fatigue, anorexia
- Neuritic pain, fasciculation at site of virus entry. Anxiety, irritability, nervousness, insomnia and depression are other features

Acute Neurological Phase

This phase is characterized by the following features:

- Hyperactivity with bouts of bizarre behaviour, agitation, seizures appearing between apparently normal periods
- This hyperactivity may be precipitated by external stimuli
- Important feature is hydrophobia, i.e. choking, gagging and fear on attempts to drink water despite intense thirst. It happens in acute neurological phase of rabies
- Patient can swallow dry solids but not liquid
- Attempt to drink causes painful spasms of pharynx—larynx producing choking
- Patient starts dreading even sight and sound of water
- Generalized convulsions may follow and can cause death due to respiratory arrest
- In some patients, instead of hyperactivity paralytic features dominate

Coma and Death

Acute neurological phase may be followed by coma and death.

Mention the methods by which rabies can be diagnosed in a laboratory?

Specimens

- Antemortem—corneal smears, skin biopsy from nape of neck, saliva, CSF
- Postmortem—impression smears of brain

Processing of Specimen

Ag Detection

Immunofluorescence—monoclonal Ab conjugated with fluorescent tag is used for detection of Ag.

Detection of Nucleic Acid

- RT-PCR
- Dot blot hybridization
- Probes

Detection of Ab

In serum and CSF by ELISA.

Virus Isolation

- Animal inoculation
 - Mouse is preferred
 - Intracerebral inoculation is preferred by using specimens
 - Brain is examined after death for Negri bodies and immunofluorescence
- Cell culture
 - Inoculated on:
 - (a) Vero monkey kidney cells
 - (b) Human diploid lung fibroblasts
 - (c) Baby hamster kidney
 - Virus is identified by immunofluorescence or neutralization with antisera

Postmortem Diagnosis

- Demonstration of Negri bodies—brain smears from hippocampus, brain stem, cerebellum are stained by Seller's technique (basic fuchsin and methylene blue alcohol)—show intracytoplasmic, 3–27 μ , oval, eosinophilic inclusions with basophilic granules
- Immunofluorescence (by using brain smears)

Describe the prophylactic measures available for preventing the spread of rabies virus in an individual bitten by a rabid animal.

Prophylaxis for rabies virus infection includes the following measures:

1. Local treatment
2. Active prophylaxis
3. Passive prophylaxis

Local Treatment

- Prompt cleaning with soap and water
- Prompt cauterization
- Disinfection with cetavlon or iodine
- In severe wounds—infiltration of antirabies serum
- Tetanus toxoid
- Antibiotics
- Postpone suturing

Active Prophylaxis**Antirabies Vaccines****Neural vaccines**

1. **Semple vaccine**—5% sheep brain inactivated with phenol
2. **Beta-propiolactone (BPL) vaccine**—It is modified Semple vaccine in which the virus is inactivated with BPL instead of phenol. Routinely used in India
Advantages: Smaller doses are required and it is more immunogenic than Semple vaccine.
3. **Infant brain vaccine**—encephalitogenic factor is scanty or absent in neural tissues of newborn animals, hence it is prepared by using infant rabbit or mouse or rat brain. It is not used in India.

Advantages

They are cheap, hence used in developing countries.

Disadvantages

They are encephalitogenic, hence not used in developed countries but still used in developing countries. They are poor immunogens.

Nonneural vaccines

1. **Egg vaccines** – These vaccines are not in use
 - a. Duck egg vaccine
 - b. Chick embryo vaccine, e.g. low egg passage and high egg passage
2. **Tissue culture vaccines**
 - a. Human diploid cell culture vaccine
 - b. Purified chick embryo cell vaccine
 - c. Purified Vero cell vaccine

Advantages

They are highly antigenic, effective, safe and routinely used.

Disadvantages

High cost

Subunit vaccine—In experimental stage

Postexposure Prophylaxis Suggested by WHO

WHO suggested the guidelines for postexposure prophylaxis by considering risk and type of exposure (Table 66.2).

Postexposure Prophylaxis with Neural Vaccines (Table 66.3)

- Vaccine is given subcutaneously on anterior abdominal wall depending on the level of risk of infection (Table 66.4)
- Protection lasts for 6 months

Table 66.2 WHO guidelines for postexposure prophylaxis of rabies

Category of risk	Type of exposure	Prophylaxis level
Class I	Touching, feeding, licks on intact skin	Not required
Class II	Licks on broken skin, scratches or abrasions without bleeding	Start vaccine, discontinue if animal is well after 10 days
Class III	Transdermal bites, scratches	Rabies Ig + vaccine, discontinue if animal is well after 10 days

Table 66.3 Neural vaccines for postexposure prophylaxis

	Semple vaccine	BPL vaccine
Class I	2 ml × 7 days	2 ml × 7 days
Class II	5 ml × 14 days	3 ml × 10 days
Class III	10 ml × 14 days	5 ml × 10 days

Class I–Class III refer to the categories of risk level (see Table 66.2).

BPL = Beta propiolactone.

Table 66.4 Degree of risk for neural vaccination in rabies

Class I Mild risk	
1.	Licks or direct contact with saliva on definitely remembered cuts on all parts except head, neck, face and fingers
2.	Licks on intact mucous membrane or conjunctiva
3.	Scratches or bites, which have raised epidermis on all parts except head, neck, face and fingers
4.	Consumption of unboiled milk or handling flesh of rabid animals
Class II Moderate risk	
1.	Licks on definitely remembered cuts on fingers
2.	All bites on fingers not more than half centimeter long (Nonlacerated and nonpenetrated)
3.	Bite or scratches on all parts except head, neck, face and fingers—drawn blood or with teeth marks or extensive laceration
Class III High risk	
1.	Licks on definitely remembered cuts on head, neck and face
2.	All bites and scratches on head, neck and face
3.	All bites on fingers more than half centimeter long, lacerated
4.	All bites penetrating skin drawing blood with five or more teeth marks
5.	Jackal or wolf bite
6.	Class II not received treatment within 14 days of exposure
7.	All bites with extensive lacerated wounds on any part of body

✓ **Postexposure Prophylaxis using Cell Culture Vaccine**

- Schedule: Six doses at 0, 3, 7, 14, 30, 90 days (last dose is optional only for those receiving Ig) protection lasts for five years. In this period if exposure occurs one or two boosters are required
- Route: Intramuscular or subcutaneous
- Dose: 0.5–1 ml

✓ **Preexposure Prophylaxis using Cell Culture Vaccine**

- Vaccine used is cell culture vaccine
- Schedule: Three doses at 0, 7, 21 days and booster at 1 year and then every 5 years
- Route: Intramuscular or subcutaneous
- Dose: 0.5–1 ml

✓ **Passive Prophylaxis**

- It is required only in severe wounds on face, neck, thorax and arms where incubation period is less
- Human rabies immunoglobulin—20 IU/kg body weight
- Half of it is infiltrated around wound and half is given intramuscularly

67

Chapter

Hepatitis Viruses

■ Enumerate the hepatitis causing viruses.

Many viruses cause hepatitis, of these the following six are commonly described as "Hepatitis viruses".

1. Hepatitis A virus (HAV)
2. Hepatitis B virus (HBV)
3. Hepatitis C virus (HCV)
4. Hepatitis D virus (HDV, delta agent)
5. Hepatitis E virus (HEV)
6. Hepatitis G virus (HGV)

Apart from these viruses, there are other viruses causing inflammation of the liver but are not called hepatitis viruses. These include:

1. Yellow fever virus
2. Epstein-Barr virus
3. Cytomegalovirus
4. Herpes simplex virus
5. Varicella-zoster virus
6. Measles virus
7. Rubella virus
8. Coxsackie virus
9. Lassa fever virus
10. Marburg virus

These viruses are not included in the category of viral hepatitis.

■ What are the morphological and cultural features of hepatitis A virus?

Morphological Features (Fig. 67.1)

- Hepatitis A virus is a typical enterovirus classified in picornaviridae family
- Size: 27 nm
- It has a single-stranded RNA genome surrounded by nonenveloped icosahedral nucleocapsid
- It replicates in the cytoplasm of cell
- It was originally designated as "enterovirus 72", but due to its unique features it is now recognized as the prototype of a new genus *Hepatovirus*
- It has only one serotype and there is no antigenic relationship to HBV or other hepatitis viruses

Cultural Features

- It is the only hepatitis virus, which can be cultivated *in vitro*
- It can be grown in some human and simian cell cultures—No CPE

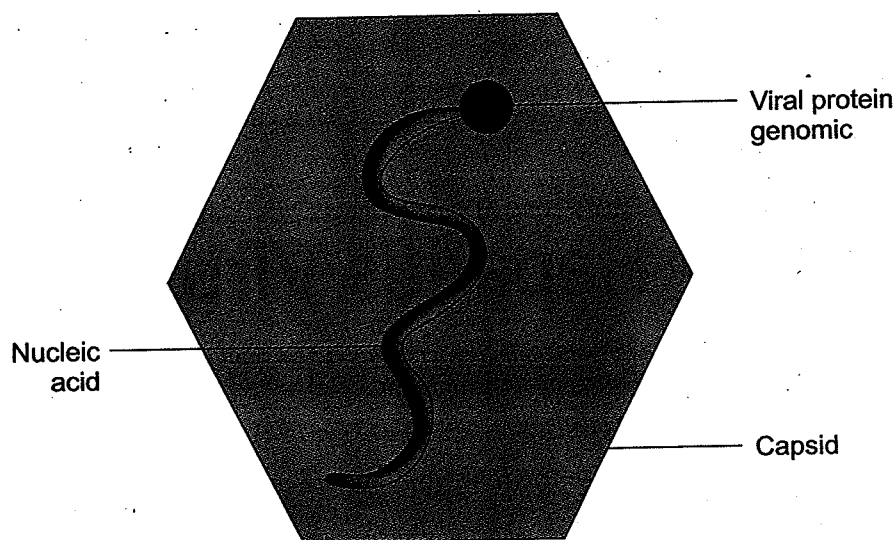


Fig. 67.1 Hepatitis A virus.

■ **Describe 'resistance' of hepatitis A virus.**

- Hepatitis A virus is resistant to heat, can resist 60°C for 1 hour
- It is resistant to ether and acid at pH 3
- It can be destroyed by boiling for 5 minutes, autoclaving and by ultraviolet radiation
- It can be inactivated by 1:4000 formaldehyde at 37°C for 3 days and chlorine 1 ppm for 30 minutes
- It is not affected by anionic detergent
- It survives prolonged storage at 4°C or below

■ **Briefly describe the pathogenicity of hepatitis A virus.**

- It causes infectious hepatitis
- HAV is transmitted by oral route (ingestion). Humans are the reservoirs of infection
- Infection occurs by ingestion of contaminated water or food grown in polluted water and eaten raw
- Common in children and young adults
- The virus multiplies in the intestinal epithelium and reaches the liver by haematogenous spread—infests hepatocytes

✓ ■ **What are the clinical features of hepatitis A virus infection (infectious hepatitis)?**

Clinical Features of Infectious Hepatitis

- Incubation period: 2–6 weeks
- Majority of infections are asymptomatic
- Infection occurs in following two stages:
 1. **The prodromal stage or preicteric stage**—characterized by fever, anorexia, nausea, vomiting and liver tenderness. The symptoms usually subside with the onset of icteric stage
 2. **The icteric stage**—characterized by jaundice. Most cases resolve spontaneously in 4–6 weeks
- Rarely—a rapidly fatal fulminant hepatitis may occur

- The disease is milder in children, in whom many infections may be anicteric
- Mortality—very low—with most of the deaths occurring in adults
- It occurs sporadically or as outbreaks, which may be caused by contaminated food, water or milk. Shellfish has been known to be responsible for outbreaks. Domestic and institutional spread of infection among children is common. Overcrowding and poor sanitation favour its spread
- HAV is rarely transmitted via blood (parenteral transmission) because the level of viraemia is low and chronic infection does not occur

✓ **Write a short note on laboratory diagnosis of hepatitis A virus infection.**

Demonstration of Virus

- Virus can be visualized by immunoelectron microscopes in faeces during the late incubation period and the preicteric phase
- As HAV usually remains associated with cell, it can be detected by immunofluorescence during its excretion in faeces

Culture

Isolation is possible but difficult as it is not possible to grow virus routinely from faeces of patients.

Detection of Ab

- Detection of IgM Ab is the most important test
- A four-fold rise in IgG Ab titre can also be used for diagnosis
- Demonstration of IgM indicates current or recent infection, while IgG indicates recent or remote infection
- ELISA—kits for detection of IgM and IgG Abs are available

■ How would you treat hepatitis A virus infection?

No antiviral therapy is available for treating hepatitis A virus.

■ What measures should be taken to prevent the spread of hepatitis A virus infection?

Hepatitis A virus infection can be prevented in the following ways:

1. Improved sanitary practices
2. Prevention of faecal contamination of food and water
3. Disinfection of water by 0.5% sodium hypochlorite solution in a dilution of 1:10 (chlorine bleach) for 30 minutes inactivates HAV
4. Specific passive prophylaxis—administration of pooled human immunoglobulin (IgG) intramuscularly before exposure to infection or in early incubation period can prevent or mitigate (attenuate) clinical illness
5. One attack of HAV gives life-long immunity. A safe and effective formalin inactivated, alum conjugated vaccine containing HAV, grown in human diploid cell culture is available. Two doses intramuscularly offer protection for 10 to 20 years

■ To which family does hepatitis B virus (HBV) belong? Mention its properties.

Family of Hepatitis B Virus

HBV causing serum hepatitis belongs to the family Hepadnaviridae.

Properties of Virus (Dane Particle)

- HBV is a complex double-layered sphere
- Size: 42 nm in diameter
- The outer surface or envelope contains hepatitis B surface Ag (HbsAg) and surrounds a 27 nm inner dense core that contains hepatitis B core Ag (HbcAg)
- The core (nucleocapsid) possesses icosahedral symmetry
- Inside the core is a genome—a single circular double-stranded DNA with DNA dependent-DNA polymerase. A complete hepatitis B virion (Fig. 67.2) is also known as **Dane particle** (Dane 1970)
- Under electron microscope, sera from HBV hepatitis patients show three types of particles:
 1. Spherical particle approximately 22 nm in diameter—most abundant
 2. Tubular or filamentous form 22 nm in diameter and of varying length. The spherical and tubular particles are antigenically identical and are the surface subunits of HBV.
 3. Dane particle—a complete HBV (Fig. 67.3)

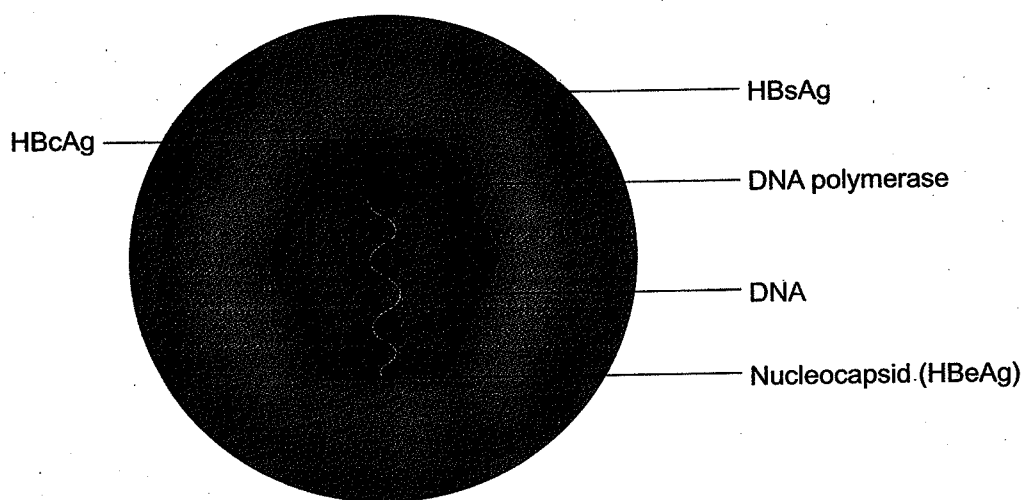


Fig. 67.2 Hepatitis B virus.

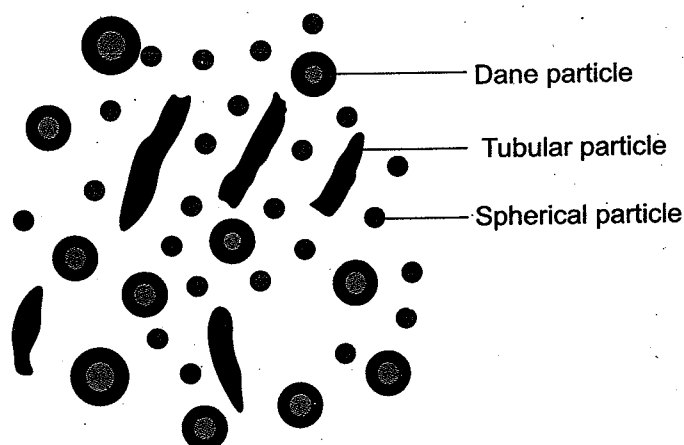


Fig. 67.3 Different types of particles of hepatitis B virus.

✓ Describe the antigenic structure of hepatitis B virus.

- Hepatitis B virus has two major Ags:
 1. The surface Ag-HBsAg
 2. The core Ag-HBcAg
- Also contains nucleocapsid—Ag-HBeAg.

HBsAg

- It is also known as Australia Ag (Blumberg 1965)
- It contains two different antigenic determinants:
 - A group-specific antigenic determinant—a
 - Two pairs of type-specific Ags—d–y and w–r. Only one member of each pair is present at a time
- HBsAg on the basis of type-specific pairing can be divided into four types:
 1. adw is worldwide in distribution
 2. adr in Asia
 3. ayw in Africa, India, Russia
 4. ayr in Africa, India, Russia
- Additional surface Ags such as q, x, f, t, j, n and g have been described, but they have not yet been characterized.

HBcAg

It is a single Ag type, found only in Dane particle and free 28 nm core particle.

HBeAg

- It is a soluble nucleocapsid protein Ag
- It has two subdeterminants—HBeAg1 and HBeAg2

■ How can hepatitis B virus be cultured?

- HBV cannot be cultivated *in vitro*
- Virus and its protein can be obtained from cell lines transfected with HBV DNA
- HBV proteins can be cloned in yeasts and bacteria

■ Describe 'resistance' of hepatitis B virus.

- HBV survives at 60°C for 60 minutes but gets inactivated at 100°C for 5 minutes
- It remains viable after desiccation and storage at 25°C for one week
- It is inactivated by formaldehyde (1:4000) and glutaraldehyde (2%)
- HBsAg is not destroyed by ultraviolet irradiation of plasma or any other blood product

■ How is hepatitis B virus transmitted?

- Transmission of hepatitis B virus predominantly occurs by parenteral route—virus enters by the percutaneous route by accidental inoculation of blood, serum, blood products or other body fluids during medical, surgical or dental procedures
- Because HBV is present in almost every body fluid, it can be transmitted by other means such as
 - Sexual contact—semen and vaginal secretions
 - Kissing—oral fluids
 - Intravenous drug abuse and tattooing
- Perinatal transmission from infected mother to newborn because of contamination of mucous membranes with mother's blood during birth is also possible

■ What are the possible consequences of hepatitis B virus infection?

- Infection with HBV may result in
 - Subclinical infection with or without HBsAg in blood
 - Subacute hepatic necrosis, which may lead to death
 - Chronic active hepatitis leading to cirrhosis
 - Chronic persistent hepatitis
 - Carrier state with minimal or no liver damage
 - Primary hepatocellular carcinoma
- The outcome of clinical infection depends up on various factors, such as age, sex, genetic background, physiologic state and immune response of host and virus strain, dose, route of inoculation. Cofactors such as drugs play role in determining severity and chronicity of the disease

■ Mention the clinical features of hepatitis B virus infection (serum hepatitis).

Clinical Features of Serum Hepatitis

- Clinically, hepatitis B viral disease is similar to HAV, but is usually more severe and protracted
 - Its onset is slow, usually insidious with long incubation period, 2–6 months
 - Fever is less common and of low grade
 - Jaundice is rare in children but often seen in adults
 - Extrahepatic manifestations are common. These include:
 - Rash
 - Arthralgia
 - Polyarteritis nodosa
 - Glomerulonephritis
 - Serum sickness
- } believed to result from Ag–Ab combination *in vivo*
- In 10–20% adults and 35% children, HBsAg persists in blood for extended period, but few of them become chronic carriers
 - There are two types of carriers:
 1. **Super carriers:** Highly infectious, show presence of HBeAg and high titre of HBsAg and DNA polymerase in blood. HBV may also be demonstrated in blood. Very minute amount of blood can transmit infection.
 2. **Simple carriers:** More common, show no HBeAg and low level of HBsAg in blood. HBV and its DNA polymerase in blood is absent. Transmission is possible only when the large volumes of blood or serum are transferred.

■ Describe the laboratory diagnosis of hepatitis B virus infection.

- The diagnosis of HBV infections can be achieved by detection of HBV Ags and Abs
- A variety of serological tests are available to diagnose:
 - Acute and chronic infections
 - The carrier's state and to assess
 - Degree of infectivity
 - Prognosis
 - Immune status of the individual
- A typical serologic profile of HBV associated markers is as follows:
 1. **HBsAg:** It is the first specific marker to appear in blood after infection, becomes detectable about a month after exposure to infection. Peak levels are seen in preicteric phase and disappears with recovery of clinical disease, but may persist for years in carriers.
 2. **Anti-HBs:** Abs to HBsAg appear within weeks after the disappearance of HBsAg and persist for years.

3. **HBcAg:** It is not detectable in serum, but can be demonstrated in liver cells by immunofluorescence test.
4. **Anti-HBc Ab:** It appears in a week or two after the appearance of HBsAg. It is the earliest Ab to appear. Two types of Abs are:
 - **Anti-HBc—IgM**—initially—indicates recent infection
 - **Anti-HBc—IgG**—Later on—persists lifelong, hence indicator of prior infection
5. **HBcAg:** It appears with HBsAg and disappears within few weeks. It is an indicator of active intrahepatic viral replication and presence of HBV DNA, virions and DNA polymerase in blood—highly infectious and persists for several months (Table 67.1).
6. **DNA Polymerase:** This is observed transiently during preicteric phase of illness.

Table 67.1 Serological profile and HBV associated markers

Clinical stage	Markers					
	HBsAg	HBeAg	HBV DNA	Anti-HBs	Anti-HBe	Anti-HBc
Incubation period	+	+	+	—	—	—
Acute infection	+	+	+	—	—	IgM
Chronic infection	+	+	+	+	—	IgG
Carrier state	+	—	—	—	+	IgG
Past infection	—	—	—	+	—	IgG
Immunization	—	—	—	+	—	—

+ = positive, — = negative.

Ag Detection

- Agar gel diffusion—simple, cheap, reliable but time-consuming
- Counter current immunoelectrophoresis—rapid and more sensitive
- Complement fixation test—more sensitive than counter current immunoelectrophoresis (CIEP)
- Passive haemagglutination test
- Reverse passive haemagglutination test
- Latex agglutination test
- Immune adherence haemagglutination test
- ELISA
- RIA

Detection of Virus and Nucleic Acid

- Demonstration of virus under the electron microscope
- PCR—highly sensitive test for detection of HBV DNA in serum

Write prophylaxis for hepatitis B virus infection?

- Hepatitis occurs following blood transfusion, injection of blood products, unsterile invasive procedures and contact with infected body fluids in drug addicts, in male homosexuals, in medical and laboratory personnel handling the infected patients and blood, in mental hospitals and in infants of carrier mothers
- Prophylaxis includes:
 1. General prevention methods
 2. Immunization

General Prevention Methods

1. Health education
2. Improvement of personal hygiene
3. Strict attention to sterility
4. Screening of blood donors for HBsAg and HBeAg

Immunization

Passive immunization

- By injecting human serum globulin. The level of protection would depend on the titre of anti-HBs in the preparation
- It is preferable to use hepatitis B immunoglobulin (HBIG) prepared from sera with high titres of anti-HBs (300–500 IU intramuscularly)

Active immunization

By using vaccines. Vaccines currently available are:

1. Plasma-derived Vaccine

- It consists biochemically-biophysically inactivated HBsAg particle obtained from the plasma of screened healthy chronic carriers by plasmapheresis
- The virus particles are collected by ultracentrifugation and subjected to inactivation by pepsin digestion and formaldehyde treatment and finally formulated in an alum adjuvant
- Dose: Two doses intramuscularly at an interval of one month and booster dose at 6 months
- It is safe and immunogenic—gives good protection—for at least 3 years
- Subsequent booster doses may be required after 5 years

2. Recombinant Yeast Hepatitis B Vaccine

- Prepared by cloning the virus DNA in yeast—genes for HBsAg are cloned
- HBsAg particles expressed on yeast cells are extracted and purified for use of vaccine
- It is immunogenic—induces good Ab response, safe and free from side-effects
- Dose: Three doses at 0, 1 and 6 months intramuscularly

■ How is hepatitis B virus infection treated?

- There is no specific antiviral treatment
- Interferon-alpha alone or in combination with lamivudine or famciclovir may be beneficial in some chronic cases

■ Write a short note on hepatitis C virus (HCV).

Properties of Virus

- HCV is a small, 50–60 nm, virus with a positive sense single-stranded RNA virus belonging to the family Flaviviridae
- It is an enveloped virus—carrying glycoprotein spikes
- It shows considerable genetic and antigenic diversity. Six different genotypes and many subtypes have been identified
- It has not been grown in culture, but cloned in *E. coli*

Pathogenesis

- Source of infection—carriers

- Infection occurs by percutaneous exposure to blood and plasma derivatives
 - Needle stick injuries, use of contaminated needles and syringes, sharing of needles by intravenous drug users, transfusion of blood and blood products, tattooing and skin piercing are the common methods of transmission
 - Sexual transmission, and mother to neonatal transmission have also been reported

Clinical Features

- Incubation period: 5–12 weeks
- Majority of cases are asymptomatic/subclinical
- Clinically the disease is less severe with milder symptoms
- Jaundice is—absent or less marked
- About 50–80% patients—develop chronic hepatitis and slowly progressive liver damage
- Clinically it may remain silent for years or even decades
- Cirrhosis and hepatocellular carcinoma may develop after 20–30 years

Laboratory Diagnosis

- **Detection of viral antigen**—by immunofluorescence of blood or biopsy specimens
- **Detection of Abs**—by ELISA and chemiluminescence assay and confirmation by recombinant immunoblot assay
- **Rapid assays**—immunochromatography based assay, which gives results within an hour
- **Detection of nucleic acid (HCV RNA)**—by RT-PCR, b-DNA signal amplification and transcription mediated amplification

Treatment

Interferon—alpha alone or in combination with ribavirin may be useful.

Prophylaxis

- No vaccine is available
- General control measures against HBV are applicable to HCV, which include safe blood and safe injection

■ Write a short note on hepatitis D virus (HDV).

- HDV was considered a defective virus that needs HBV as a helper for replication and expression
- Now it is considered as a **satellite hepatitis B virus**
- It was first detected in Italy (1977) and named **Delta agent**

Properties of Virus

- HDV is spherical
- Size: 35–38 nm in diameter
- It consists of a single-stranded small circular molecule of RNA genome and delta Ag or HDaAg—a nucleoprotein encoded by RNA, and the outer envelope of HBsAg encoded by HBV genome indicating that HBV is necessary for the production of HDV virions
- HDV replicates in the nucleus of infected cells and it never appears with HBcAg
- Only one serotype is recognized

Pathogenesis

- HDV enters by parenteral route
- As presence of HBV is must for the replication of HDV, HDV infection occurs only in the presence of HBV

- HDV infection is possible in three situations:
 1. Simultaneous infection with both HBV and HDV (co-infection)
 2. Superinfection by HDV in patients already suffering from HBV
 3. Superinfection of asymptomatic carrier of HBV by HDV

Clinical Features

- Incubation period: 2–12 weeks
- HDV infection results in hepatitis of increased severity than the HBV infection and may progress to fulminant hepatitis

Laboratory Diagnosis

- **Detection of delta Ag in the nuclei of hepatocytes**—by direct immunofluorescence test. Delta Ag can also be visualized in the liver biopsy specimens by indirect immunoperoxidase staining
- **Detection of nucleic acid (HDV RNA)**—by hybridisation using a radiolabelled probe
- **Ab detection**—by ELISA and RIA. Anti-HDV IgM appears after 2–3 weeks and is soon replaced by IgG

Prevention and Treatment

- No specific therapy is available
- No specific immunoprophylaxis is known
- Immunization with HBV vaccine is effective as HDV is unable to infect persons immune to HBV

■ Write a short note on hepatitis E virus (HEV).

HEV belongs to the family Calciviridae and genus *Calcivirus*.

Properties of Virus

- Size: Small—27–38 nm in diameter
- Shape: Round
- Genome: Single-stranded RNA (positive strand)
- Capsid: Icosahedral capsid without envelope

Pathogenesis

- Water contaminated with sewage is the reservoir
- Infection occurs by ingestion of contaminated drinking water

Clinical Features

- Incubation period: 2–8 weeks
- Clinically, the disease resembles hepatitis A
- It is generally mild and self-limited and does not lead to chronic hepatitis, cirrhosis, cancer or carrier state. Fulminant hepatitis is rare except in pregnant women
- It is predominantly a disease of young population. It usually occurs in the form of explosive outbreaks but may also occur in endemic or sporadic form
- It is the major public health problem in developing countries

Laboratory Diagnosis

Diagnosis can be achieved by:

- **Direct Demonstration of virus particles** in faeces using monoclonal Abs under the electron microscope
- **Detection of Abs**—IgM or IgG Abs to HEV by ELISA or Western blot test
- **Detection of HEV RNA**—can be detected in faeces or acute phase sera of patients

Treatment

No specific therapy is available.

Prophylaxis

- No vaccine is available
- The disease can be prevented by
 1. Provision of safe drinking water
 2. Improving standards of sanitation

■ Comment on hepatitis G virus (HGV). Describe its pathogenicity.**Features of Hepatitis G Virus**

- Hepatitis G virus is a new flavivirus-like agent, isolated from a patient with chronic hepatitis in 1994 .
- This agent is different from HCV and is named HGV
- It is a RNA virus

Pathogenesis

- The virus enters the body through blood transfusion and by other parenteral routes
- HGV RNA has been found in patients with acute, chronic and fulminant hepatitis, but its role in hepatitis not clear
- The virus has also been found in haemophiliacs, patients with multiple transfusion, blood donors and intravenous drug users

68

Chapter

Oncogenic Viruses

■ What are oncogenic viruses?

Viruses that produce tumours in hosts or induce malignant transformation of cells in culture are called oncogenic viruses.

■ Name the families and viruses responsible for causing cancer in humans.

Human Oncogenic Viruses

1. DNA Viruses

- Papovaviridae
 - Papilloma virus
 - Polyoma virus
- Poxviridae
 - *Molluscum contagiosum*
- Adenoviridae
 - Adenoviruses
- Herpesviridae
 - Herpes simplex virus 1
 - Herpes simplex virus 2
 - Epstein-Barr virus
 - Cytomegalovirus
- Hepadnaviridae
 - Hepatitis B virus

2. RNA Viruses

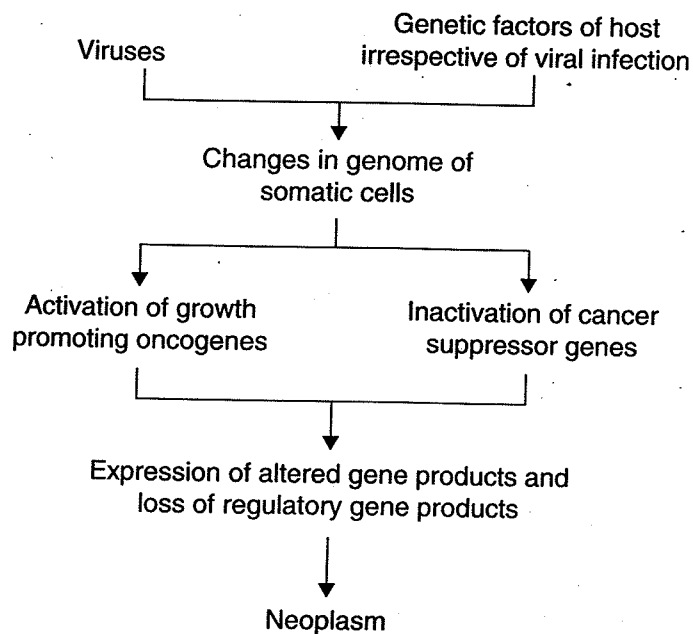
- Retroviridae: HTLV-1, HTLV-2
- Flaviviridae: Hepatitis C virus

■ Diagrammatically represent the mechanism by which neoplasm develops?

The ways in which neoplasm can develop are depicted in Flowchart 68.1.

■ What are oncogenes and how do they originate in a normal cell? What is their role in causing cancer?

- Viral oncogenes are the genes that stimulate transformation of a normal cell into a malignant cell
- Genes resembling viral oncogenes are present in normal cells as well as in malignant cells
- Oncogenes present in a malignant cell are called **cellular oncogenes** while similar genes in a normal cell are called **proto-oncogenes**



Flowchart 68.1 Mechanism of neoplasm development.

- Viral oncogenes originate from proto-oncogenes by recombination of viral and cellular gene
- **Role of oncogenes:** Oncogenes code for proteins that are involved in the process of malignant transformation of normal cells

■ How can oncogenes be studied?

Oncogenes are studied by **transfection**. Certain mouse fibroblast cell lines such as NIH 3T₃ can take up foreign DNA, incorporate them into their genome and express them. This type of gene transfer is known as transfection.

By this method, DNA extracted from human tumour cell is shown to transform 3T₃ cells. Such transforming genes have been shown to be identical with cellular oncogenes.

■ Mention the properties of transformed cells (cancer cells).

Properties of transformed cells:

1. **Changes in morphology**
 - Change in cell morphology
 - Chromosomal alteration
2. **Changes in cell metabolism**
 - Increased rate of growth
 - Increased metabolic activity
3. **Changes in growth characters**
 - Loss of cell contact inhibition
 - Formation of microtumours
 - Capacity to divide indefinitely in cultures
4. **Changes in antigens**
 - Appearance of new viral specific tumour Ags
 - Loss of surface Ags

■ Name the oncogenic viruses and the type of cancers they cause.

Viruses and their associated cancers are as follows:

1. Human papilloma virus—cervical, vulvar and penile squamous cell carcinoma
2. Epstein-Barr virus (EBV)—nasopharyngeal carcinoma, Burkitt's lymphoma and B-cell lymphoma
3. HSV-2—B-cell lymphoma, cervical cancer
4. Hepatitis B virus (HBV)—hepatocellular carcinoma
5. Hepatitis C virus (HCV)—hepatocellular carcinoma
6. Human T-lymphotropic virus (HTLV)—T-cell leukaemia

69

Chapter

Human Immunodeficiency Virus (HIV)

■ Which virus causes acquired immunodeficiency syndrome (AIDS)?

- Human immunodeficiency virus is the causative agent of AIDS
- It belongs to the family Retroviridae

■ When was HIV first observed? Who isolated it and what was it called earlier?

- HIV was first observed in 1981 in New York, USA
- In 1983—retrovirus was isolated by Luc Montagnier and colleagues from Pasteur Institute, Paris
- It was first labelled as LAV (lymphadenopathy-associated virus) and HTLV-III (human T-cell lymphotropic virus-III)
- It is called HIV (human immunodeficiency virus) by the International Committee on Virus Nomenclature

✓ ■ Describe the morphological features of HIV.

Morphological Features

- HIV is spherical, enveloped RNA virus, 90–120 nm in diameter
- Nucleocapsid—has outer icosahedral shell and inner cone-shaped core
- Genome—consists of two identical single-stranded (positive sense) RNA copies and **reverse transcriptase enzyme**
- Envelope—is lipoprotein in nature, having matrix protein and host cell derived lipid bilayer envelope from which glycoprotein peplomers project (Fig. 69.1)

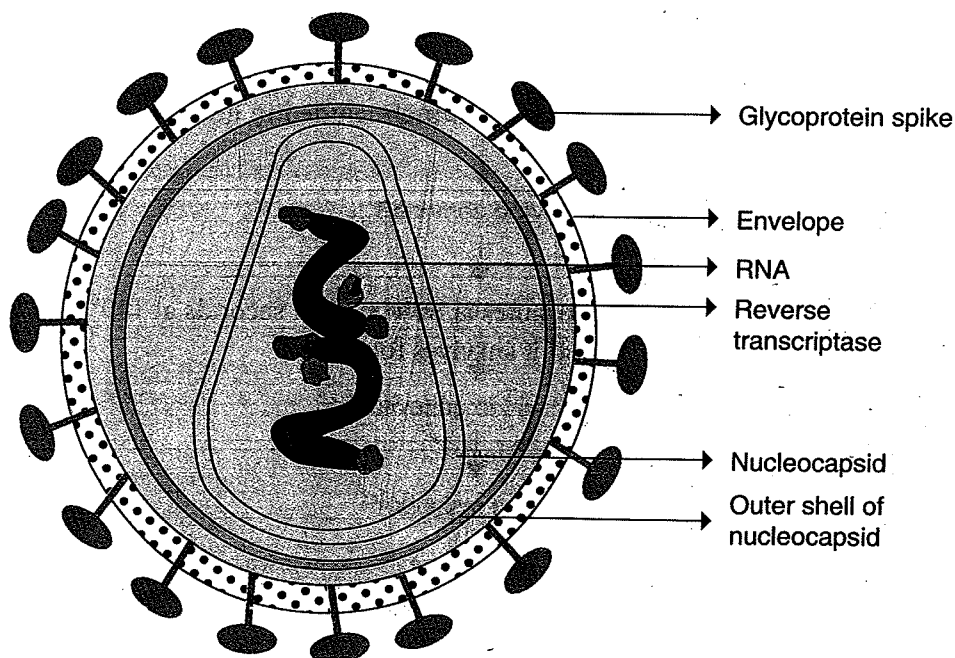


Fig. 69.1 Human immunodeficiency virus (HIV).

■ Describe the genes and antigens of HIV.

The genome of HIV consist of structural and nonstructural genes. The products of these genes act as antigens.

Structural Genes

1. Envelope Ags
 - Spike Ag—gp 120
 - Transmembrane pedicle Ag—gp 41
2. Gag Ag—core and shell Ag
 - Shell—nucleocapsid Ag—gp 18
 - Core—Major Ag—p24
 - Other Ag—p55, p15
3. Pol Ag—code for polymerase p31, p51, p65

Nonstructural and Regulatory Genes

1. *tat* (trans activating gene)—Enhances expression of viral genes
2. *nef* (negative factor gene)—Regulates viral replication
3. *vif* (viral infectivity factor gene)—Influences infectivity of viral particles
4. *vpu* (in HIV I only) and *vpx* (only in HIV II)—Enhance maturation and release of progeny virus
5. *vpr*—stimulating promoter region of virus
6. LTR (long terminal repeat sequences)—Gives promoter, enhancer and integration signals

Antigenic Types and Variation

1. It exhibits frequent antigenic variation; they are frequent with envelope Ag
2. HIV has 2 antigenic types—1 and 2
3. First case of HIV-1 was reported in 1986 and HIV-2 in 1991 in India
4. HIV-2 shows 75% homology with simian HIV virus and 40% with HIV-1
5. Envelope—Ags of type 1 and 2 are different
6. HIV-2 is more virulent than HIV-1
7. HIV-1 has 10 subtypes A to J and are included in group M (for major)
8. Other subtypes, which do not fall into group M are called outlier and are included in O group (for outlier)
9. Some newer subtypes distinct from M and O are included in group N (for new)
10. In India subtype C is common

■ Describe 'resistance' of HIV.

- HIV is a delicate virus
- It is thermolabile—destroyed in 10 minutes at 50°C and in seconds at 100°C
- At room temperature—in dried blood it survives for 7 days
- It is sensitive to disinfectants such as
 - 70% ethyl alcohol
 - 0.5% lysol
 - 0.1% formalin
 - 2% glutaraldehyde
 - 0.3% hydrogen peroxide
 - 0.2% hypochlorite
 - 10% household bleach

- It is sensitive to detergents—so washing with hot water and detergents is used for decontamination of clothes
- For disinfection of medical instruments, 2% glutaraldehyde is used
- For surface disinfection—household bleach can be used

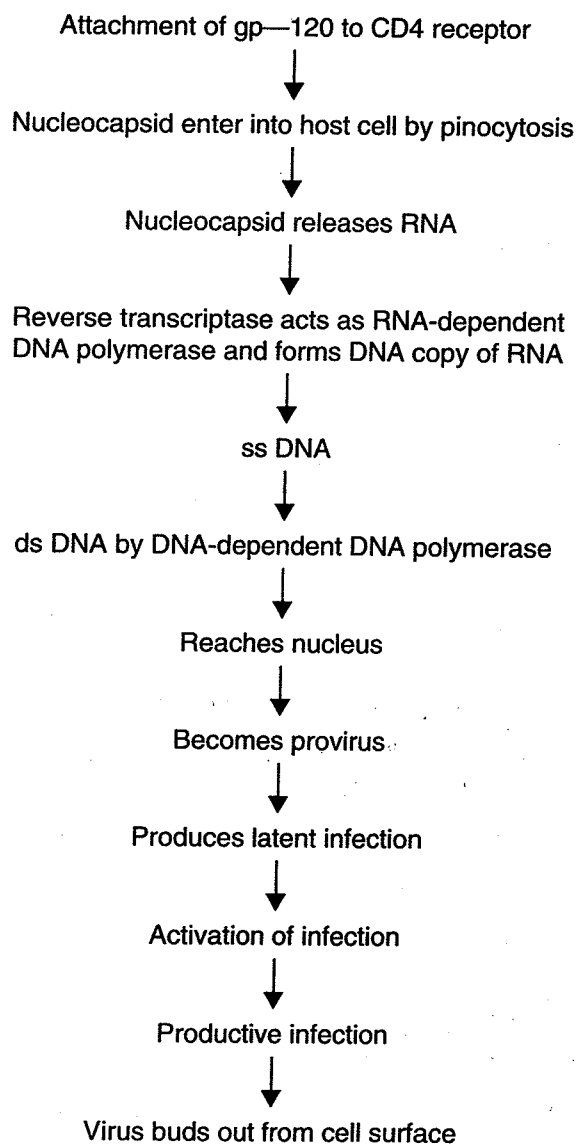
■ **Mention the routes of HIV transmission and their percent efficiency in spreading infection.**

Routes of HIV transmission occurs by

1. Blood transfusion—90%
2. Perinatal—12–40%
3. Sexual intercourse—0.1% per episode
4. IV drug use—0.5–1%

✓ ■ **Explain replication of HIV.**

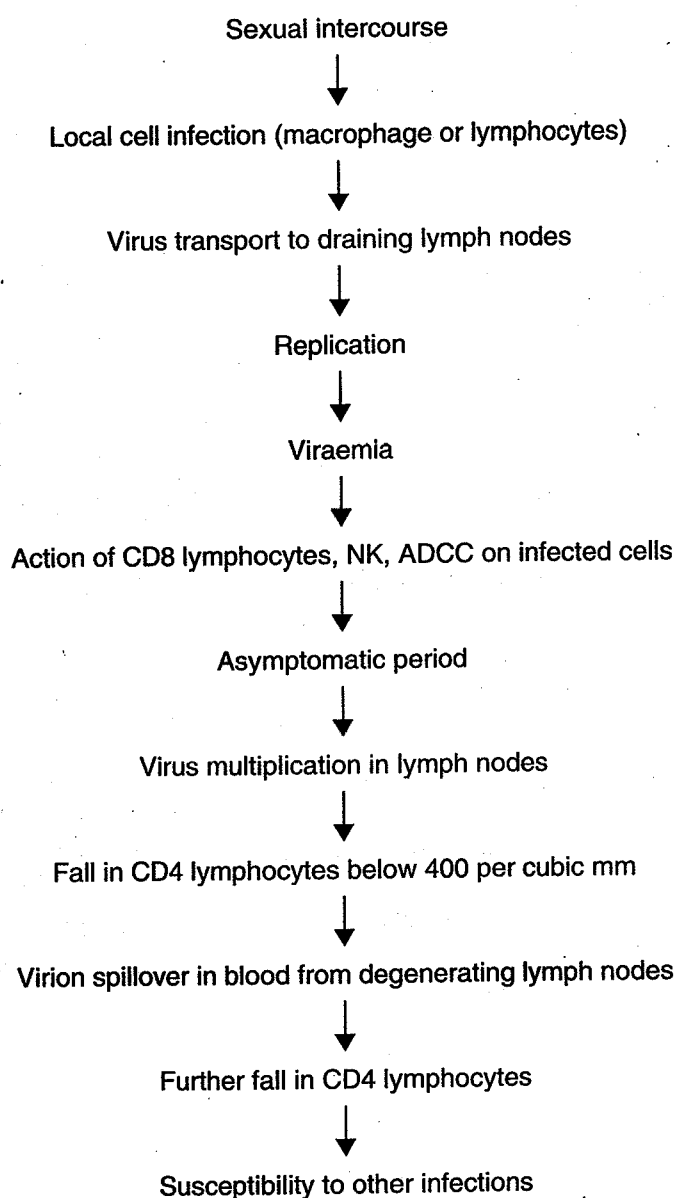
The process of HIV replication is presented in Flowchart 69.1.



Flowchart 69.1 Replication of human immunodeficiency virus.

Trace the course of disease development in case of HIV infection.

The various stages involved in the development of disease due to HIV infection are presented in Flowchart 69.2.



Flowchart 69.2 Course of disease development in HIV infection (CD = cluster of differentiation, NK = natural killer, ADCC = antibody-dependent cell-mediated cytotoxicity).

■ In HIV infection, why is there fall in CD4 lymphocytes?

Fall in CD4 lymphocytes is due to

1. Viral cytolysis of CD4 cells
2. Infected cells fuse to other noninfected cells via gp120 forming syncytia and leads to death of entire syncytium
3. Immune cytolysis of infected cell by NK, CD8, T cells, ADCC

What are the effects of fall in CD4 lymphocytes on CMI and humoral immunity?

- **Effect of fall in CD4 lymphocytes on CMI:** Infected CD4 T cells do not release normal amounts of IL-2, gamma interferon and other lymphokines, which affect CMI
- **Effect of fall in CD4 lymphocytes on humoral immunity:** Th (T helper) cell functioning is not optimal, hence it hampers in B cell function. This leads to polyclonal activation of B cells that results in hypergammaglobulinaemia

Describe the clinical features of HIV infection.

Clinical Features

The clinical course can be classified into the following four groups (Center for Disease Control and Prevention [CDC], Atlanta, USA):

CDC Classification of HIV Infection

- Group I—acute infection or seroconversion illness
- Group II—asymptomatic infection
- Group III—persistent generalized lymphadenopathy
- Group IV—other diseases
 - a. Constitutional diseases or ARC
 - b. Neurological diseases
 - c. Secondary infectious diseases
 - d. Secondary cancers
 - e. Other conditions

Acute HIV Infection

- It presents with fever, myalgia, arthralgia, headache, malaise, sore throat, diarrhoea, rash
- Viral nucleic acid can be detected, Abs are usually not found initially but found in due course, so it is called seroconversion illness

Asymptomatic Infection

This stage shows positive HIV Ab tests.

Persistent Generalized Lymphadenopathy

- It is found in 25% of the patients
- Enlarged lymph nodes, 1 cm in diameter at two or more extra inguinal sites persist for 3 months

Symptomatic HIV Infection

Opportunistic infections are found in this stage (Table 69.1).

Table 69.1 Opportunistic infections and malignancies in AIDS

1. Bacterial— <i>M. avium</i> , <i>M. tuberculosis</i> , <i>Salmonella</i>
2. Viral—Herpes simplex, cytomegalovirus, varicella-zoster
3. Fungal— <i>Candida</i> , <i>Cryptococcus</i> , <i>Aspergillus</i> , <i>Histoplasma</i> , <i>Pneumocystis carinii</i>
4. Parasitic— <i>Toxoplasma</i> , <i>Cryptosporidium</i> , <i>Isospora</i> , <i>Strongyloides stercoralis</i>
5. Malignancies—Kaposi's sarcoma, B-cell lymphoma

Describe the laboratory diagnostic methods for detecting HIV infection.

Tests for diagnosing HIV infection can broadly be categorized into two types—immunological tests and specific tests. Immunological tests are used to assess the immunological status of the patient and specific tests are used to diagnose HIV infection.

Immunological Tests

1. Total leucocyte and lymphocyte count to demonstrate leucopenia and lymphocyte count is usually less than 2000 mm^3
2. CD4^+ T cells less than 200 mm^3
3. CD4 and CD8 ratio is reversed because of decrease in CD4 cells
4. IgG and IgA—raised
5. Lymph node biopsy—abnormal

Specific Tests

The types of specific tests used in laboratory for diagnosing of HIV infection are summarized in Table 69.2.

Table 69.2 Specific tests used in laboratory diagnosis of HIV Infection

1. Ag detection test
 - ELISA
2. Nucleic acid detection
 - PCR
 - Branched DNA
 - In situ hybridisation
3. Virus isolation
 - Cocultivation with uninfected lymphocytes and Interleukin-2
4. Ab detection
 - a. Screening tests
 - ELISA
 - Rapid tests—Latex agglutination, Immunocomb II, HIV Tridot, etc.
 - b. Confirmatory tests
 - Western blot test

Ag Detection by ELISA

- It can be detected during window period (It is a seronegative stage during which no antibodies are detectable in serum in spite of infection, i.e. a period between entry of virus to appearance of detectable antibody in serum—usually 2–8 weeks, in about 25% of cases) and AIDS patients (60%)
- It can be used in early and late infections
- It becomes negative when Abs appear

Nucleic Acid Detection

- It can be used in the diagnosis of all stages
 1. Target amplification test—PCR for detection of nucleic acid, RT-PCR for quantitation of viral load
 2. Signal amplification test—branched DNA test can be used
 3. In situ hybridisation—It is method of detection of viral nucleic acid in tissues by using probes
- They are highly specific and sensitive tests but are costly and complex.

Virus Isolation

- Routinely impracticable. Feasible in research laboratories only
- Patient's lymphocytes are cocultivated with uninfected lymphocytes in presence of interleukin-2
- Presence of virus is indicated by detection of p24 Ag and reverse transcriptase activity

Detection of Abs

- Ab takes 2–6 months to appear following sexual exposure
- IgM and IgG can be detected in patient's sera
- They are detected for screening, sero-epidemiology, diagnosis and to judge prognosis, by various tests (Table 69.3)

Table 69.3 Evolution of serological markers

State of infection	p24 Ag	IgG	IgM	Western blot pattern
Acute illness	+ → -	- → +	+	Partial
Carrier (asymptomatic)	-	+	-	Full
PGL	+	+	-	Loss of p24/p55 Ab
AIDS	+	-	-	Absence of p24 Ab

PGL = persistent generalized lymphadenopathy, + = positive, - = negative, + → - = positive to negative, - → + = negative to positive.

Screening tests

- These are highly sensitive, simple to perform, can be automated but are not highly specific and may give false positive results, therefore positive reaction should be confirmed by confirmatory tests, which are highly specific
- ELISA is widely used and ideal test for screening
- **Rapid tests**—results can be obtained in less than 30 minutes. Different tests used are summarized in Table 69.4.

Table 69.4 Rapid tests used in laboratory diagnosis of HIV infection

Test	Principle	Visual Interpretation
Capillus and Serodia (using latex, gelatin or RBCs as carrier molecule)	Particle agglutination	Clumping
<ul style="list-style-type: none"> • Comb AIDS RS • Immunocomb II • HIV1 and HIV2 BiSpot 	Immuno-comb	Spot (Dip stick / comb test)
<ul style="list-style-type: none"> • HIV Tridot, HIV spot 	Immunoconcentration	Dot (dot blot assays)
<ul style="list-style-type: none"> • SD Bioline • Retrocheck HIV 	Immunochromatography	Line (lateral flow assays)

Confirmatory test**Western blot**

- In this test, strips of nitrocellulose paper are used which are blotted with proteins of HIV separated by polyacrylamide gel electrophoresis
- Strip is reacted with sera; enzyme is conjugated with antihuman globulin and then substrate is added which produces colour band where Ab reacts with specific Ag
- Position of band indicates Ag with which it reacted
- Positive test shows presence of at least 2 bands of Ags p24, gp 41, gp 120/160

■ By which methods is HIV infection monitored in a laboratory?

Laboratory monitoring of HIV infection is done by:

1. **CD4 T cell count**—counts below 500/mm³ is indication for need of antiviral therapy. Counts below 200 denote risk of serious infection

2. **RT-PCR**—helps to measure viral load
3. **Beta 2 microglobulin and neopterin**—they can be measured in serum and urine. Concentrations are low in asymptomatic infections and increase with advanced stages

■ Which HIV testing strategies are followed in India?

HIV testing strategies followed in India are:

Strategy I

- Serum tested by one screening test
- If positive—should be considered positive and if negative—considered negative

Use

It is used in blood banks for transfusion safety.

Strategy II

- Serum tested by one screening test
- If positive, retested by another test based on different Ag preparation or principle
- If positive in second test—reported as positive

Use

It is used for HIV surveillance.

Strategy III

- Serum tested by two screening tests
- If positive, retested by third test based on different Ag preparation or principle
- If positive in all three tests—reported as positive and negative in third considered equivocal and retested after 3 weeks
- If again gives equivocal results person is declared negative

Use

It is used for diagnosis

■ What treatment is recommended for HIV infection?

- Dideoxynucleoside analogues that inhibit reverse transcriptase are effective inhibitors of replication, e.g. AZT, it reduces chances of severity of opportunistic infections but resistance to drug is observed
- Other drugs are:
 - Nonnucleoside inhibitors—nevirapine, zidovudine
 - Protease inhibitors—saquinavir, zalcitabine, didanosine

■ How can infection with HIV be prevented?

Infection with HIV can be prevented in the following ways:

1. Health education regarding 'safer sex' methods
2. Health education of medical and paramedical staff
3. Health education of infected mothers
4. Screening of donors
5. Use of disposable syringes and needles
6. Avoidance of sharing syringes in drug addicts

70

Chapter

Miscellaneous Viruses

■ What are papovaviruses?

Papova are small, enveloped, DNA viruses with icosahedral symmetry.

■ Name medically important papovavirus.

- Medically important papovavirus is **papillomavirus**
- Its medical importance is:
 - It is species-specific
 - It infects squamous epithelia and mucous membranes and produces warts
 - Human papilloma virus (HPV) infects humans only

■ Name the diseases caused by human papillomavirus along with the type of the causal virus.

Following are the types of human papilloma virus with the diseases caused by them:

1. HPV Types 1, 2, 3, 4—Warts on hand and feet
2. HPV Types 6 and 11—Genital warts. These may be transmitted venereally, may also become malignant
3. HPV Types 16, 18—Uterine cervical cancer

■ Write a short note on parvovirus.

Medically important type of parovirus is Parvovirus B 19

- It is a small, 18–25 nm, nonenveloped DNA virus
- Its genome—is single-stranded DNA
- Transmission—occurs through respiratory route usually, may be through blood
- It causes respiratory infection with erythematous, maculopapular rash and arthralgia usually in children 5–10 years old
- Induces aplastic crisis in children with chronic haemolytic anaemias as in sickle cell disease
- It causes persistent anaemia in immunodeficients
- Detection of virus in blood in early stage of infection
- Ab detection in later stages

■ What is the medical significance of rubella virus?

- Rubella virus causes mild fever with macular rash and lymphadenopathy
- It is specially important because disease in pregnant women causes congenital malformation and mental retardation
- Consequences of rubella *in utero* are referred to as **congenital rubella syndrome**

■ Mention the properties of rubella virus.

- Rubella is an enveloped, RNA virus
- It is pleomorphic or spherical
- Size: about 50–70 nm in diameter with ssRNA genome
- Its envelope carries haemagglutinin peplomers
- It grows in primary and continuous cell lines such as Rabbit kidney-13 and BHK-21
- The virus produces cytopathic effects in few cell lines, while in others virus growth is identified by interference using challenge virus ECHO-11

■ Describe the infection caused by rubella virus.

Rubella virus causes **rubella**, also known as **German measles**. The disease may affect people of all age groups.

The disease can be categorized into the following two groups:

1. Perinatal Rubella

- Infection is acquired by inhalation
- Generalized rash first appears on face then spreads to neck and trunk extremities (spare palm and sole)

2. Congenital Rubella

- Fetal damage is related to a stage of pregnancy, earlier infection in pregnancy causes more damage to fetus
- It may cause abortions, fetal death or congenital malformations
- Congenital malformations—Classical congenital rubella syndrome constitutes cardiac defects, cataract and deafness
- Others symptoms
 - Hepatosplenomegaly
 - Thrombocytopenic purpura
 - Myocarditis
 - Bone lesions
 - Developmental retardation of fetus

■ Briefly describe the laboratory diagnosis of rubella infection.

Rubella in pregnancy needs confirmation. It is done by virus isolation and serology.

Specimens

Blood, throat swab, nasopharyngeal swab.

Isolation

Virus can be cultivated in primary and continuous cell lines and growth of virus is detected by detection of virus Ag by immunofluorescence.

Serology

- Haemagglutination inhibition
- ELISA—IgM and IgG detection
 - Presence of IgG denotes immunity
 - To confirm recent rubella infection either rise in titres of IgG in two samples collected 10 days apart or IgM must be demonstrated in a single sample

■ **Name the viruses that cause diarrhoea.**

Viruses that cause diarrhoea are as follows:

1. Rotavirus
2. Norwalk virus
3. Adenovirus
4. Coronavirus
5. Astrovirus

✓ **Write a short note on Rotavirus.**

Rotavirus is the major cause of diarrhoea in infants.

Properties

- It is an RNA virus with double-stranded RNA
- It is spherical with icosahedral symmetry about 65 nm in diameter
- The virus has the characteristic wheel-like appearance

Pathogenicity

- Infection is acquired by ingestion of contaminated food and water or directly from contaminated fingers
- Rotavirus gastroenteritis is observed in infants aged 6–24 months. Stool is green yellow without blood and mucus
- Diarrhoea and vomiting can lead to severe dehydration
- It is usually associated with fever

Laboratory Diagnosis

Specimen

Stool sample.

Collection

Collected in dry container, emulsified in buffered saline. Rectal swab is collected and immersed in viral transport medium.

Direct demonstration

Electron microscope, immune electron microscope.

Virus Ag detection in stool

- CFT
- CIEP
- ELISA
- Passive agglutination

Ab detection in blood

IgM and IgG detection by ELISA.

■ **Mention the general properties and pathogenicity of Norwalk virus.**

General Properties

- It is 27 nm, small, round, RNA virus
- Virus cannot be cultured

Pathogenicity

- Incubation period: 24–48 hours
- Norwalk virus is the cause of epidemic viral gastroenteritis in adults, rapid onset diarrhoea, vomiting, nausea, fever, abdominal cramps, headache and malaise are the features
- Infection is transmitted by faeco-oral route

■ How can Norwalk virus infection be diagnosed in a laboratory?

Norwalk virus infection can be diagnosed in a laboratory by the following methods:

- **Virus or antigen detection** and detection of viral genome by RT-PCR
- **Virus demonstration in stool**—by electron microscope
- **Ab detection** — by ELISA. four - fold rise of Abs in paired sera indicates recent infection

■ What are Coronaviruses? Enumerate their general properties.

These are viruses belonging to the family Coronaviridae, they cause **common cold** and **SARS** (subacute respiratory syndrome) in adults and gastroenteritis in infants.

General Properties

- Spherical in shape, 120–160 nm in diameter with helical nucleocapsid
- RNA virus with nonsegmented single-stranded RNA
- Enveloped virus with petal-shaped spikes
- Have high frequency of recombination
- Difficult to grow in cell cultures, Vero cell lines can be used

Pathogenicity

- Incubation period: 2–5 days
- Human coronavirus is the cause of common cold. Up to 30% of common colds are caused by coronaviruses
- Infection occurs by inhalation
- Coronaviral common cold is characterized by more nasal discharge and malaise (as compared to rhino viruses) with little or no fever. Cough and sore throat are generally less prominent as compared to rhinoviruses. Coronaviruses may also cause severe lower respiratory tract infections in children and aged individuals, SARS (subacute respiratory syndrome) in adults and gastroenteritis in infants

■ What are the methods by which coronavirus infections can be diagnosed in a laboratory?

Coronavirus infections can be diagnosed in a laboratory by the following methods:

- **Ag detection**—by ELISA
- **Virus demonstration**—by electron microscopy of stool
- **Nucleic acid detection**—by PCR
- **Isolation**—of virus from respiratory secretions and stool in Vero cell lines
- **Ab detection**—by passive haemagglutination and ELISA

■ What are Astroviruses?

- Astroviruses are 28–30 nm, star-shaped, single-stranded RNA viruses
- They have 8 serotypes
- They cause diarrhoeal disease in children and immunocompromised hosts

■ **Name the viruses that cause haemorrhagic fever. Add a note on Ebola virus.**

The following viruses cause haemorrhagic fever:

- Smallpox virus
- Chickenpox virus
- Measles virus
- Yellow fever virus
- Dengue fever virus
- Chikungunya virus
- Kyasanur forest disease virus
- Omsk haemorrhagic fever virus
- Crimean congo haemorrhagic fever virus
- Junin and Machupo virus (It causes South American haemorrhagic fever, belongs to Arenaviridae. Disease is zoonotic, transmission occurs through rodent excreta)
- Lassa fever virus (Source of infection is rodent excreta. Person to person transmission occurs by droplet infection)
- Marburg and Ebola viruses (Enveloped RNA viruses belonging to Filoviridae)
- Hantaan virus (It is a RNA virus, enveloped and belongs to Bunyaviridae. Source of infection is rodent excreta)

Ebola virus

- Ebola is a rare and deadly disease caused by one of the Ebola virus strain. Earlier it was called as Ebola haemorrhagic fever.
- Recently there was epidemic in 2015 in Guinea and Sierra Leone where thousands of cases and deaths were reported.
- There are five identified species of Ebola virus, four of them can cause human disease.
- The first case was found near Ebola river in Africa.
- Transmission occurs by direct contact through skin, mucous membrane with blood, body fluids of infected person
- Needle, syringes, cloths, bedding, etc. contaminated with blood and body fluids of infected patients. Also by infected wildlife such as fruit bats or primates.

Clinical features

- Incubation period – 2-21 days
- Characterized by fever, severe headache, muscle pain, weakness, diarrhoea, vomiting, abdominal pain and unexplained hemorrhages. Commonly seen in healthcare workers and individuals in close contact with patients.

Prevention and Treatment

- Stringent hygiene-hand wash and care during handling of dead body.
- Avoidance of contact with blood and body fluids of infected human beings and primates and bats.
- Vaccines are under trial
- No specific antiviral therapy is available.

■ **What are the slow virus diseases?**

Slow virus diseases are a group of infections characterized by very long incubation period, chronic degenerative neurological involvement and fatal termination.

■ What are the characteristics of 'slow viruses'?

- Slow viruses are enveloped RNA viruses, with spherical shape and diameter around 90 nm
- They are neurotropic (predilection to involve CNS)
- Diseases caused by them have a long incubation period (in years)
- The course of illness also lasts for years and has fatal termination
- Immune response does not avert disease
- They have genetic predisposition

■ Classify the slow virus diseases. Comment on features of slow virus diseases occurring in human beings.

Classification

Slow virus diseases may be classified into the following three groups:

1. Group A—includes slowly progressive infections of sheep—caused by nononcogenic lentiviruses, e.g. Visna, Maedi. Visna and Maedi are caused by closely related lentiviruses, which may be variant strains of a single virus. The virus belongs to a lentivirus group to which human immunodeficiency virus (HIV) belongs, hence AIDS has many features similar to slow virus disease

Visna

It is a demyelinating disease of sheep characterized by pareses, paralysis and death. It was eradicated by slaughtering of all infected animals in 1951.

Maedi

It is a slowly progressive fatal haemorrhagic pneumonia of sheep.

2. Group B—includes infections in animals—diseases caused are scrapie, mink encephalopathy, bovine spongiform encephalopathy (mad cow disease)

- **Infection in humans**—the diseases are: Creutzfeldt–Jakob disease (CJD), kuru
- Infective agent is proteinaceous in nature, devoid of RNA and DNA. These are known as **prions**
- It is usually resistant to physical and chemical agents
- It can be transmitted to experimental animals by oral and parenteral challenge
- The disease occurs due to proliferation of abnormal protein PrP. Its accumulation in CNS disrupts architecture and function of brain

Classical Creutzfeldt–Jakob disease (CJD)

- It is subacute encephalopathy characterized by progressive in coordination, dementia and fatal termination in a year
- Two forms of CJD are Gerstmann–Sträussler–Scheinker's syndrome and fatal familial insomnia
- CJD occurs after corneal transplant and contaminated injection of pituitary growth hormone, by cadaveric human dura grafts used in head injury
- Protein similar to scrapie PrP is present in brain tissue infected with classic CJD, so it has been speculated that agent of CJD was derived from scrapie-infected sheep and transmitted to humans by ingestion of poorly cooked sheep brain
- A variant of CJD that affected young persons (below 45 years) in Britain in 1996, raised fears of infection through eating bovine spongiform encephalopathy infected beef

Kuru

- It is a disease in humans that leads to progressive cerebellar ataxia and tremors, and terminates fatally in 3–6 months
- Incubation period: 5–10 years
- Infection is believed to have been introduced through cannibalism and maintained by the tribal custom of eating dead bodies of relatives after ritual nonsterilizing cooking

3. Group C—includes two central nervous system diseases of human beings: subacute sclerosing pan encephalitis, and progressive multifocal leucoencephalopathy

Subacute sclerosing pan encephalitis

- It is a delayed sequelae of measles virus infection
- Condition develops after many years of initial infection and is characterized by progressive deterioration of mental and motor function
- Brain cells show evidence of measles virus infection
- Strains isolated are defective
- Patient shows high levels of virus antibody in serum and CSF
- Finding Abs in CSF is characteristic

Progressive multifocal leucoencephalopathy

- It is a rare demyelinating disease in elderly persons whose immune response is impaired by immunosuppression and malignancy
- Clinically it shows deterioration of motor function, vision and speech
- Papova virus has been demonstrated by electron microscopy in brain biopsy of patients

UNIT

V

Mycology

M

R

•

71

Chapter

Introduction to Mycology

■ What do Mycology and Medical Mycology deal with?

Mycology is the branch of Microbiology that deals with the study of fungi

- Medical Mycology deals with fungi causing diseases in human beings

■ Who is the Father of Mycology?

Raymond Sabouraud is known as the Father of Mycology.

■ Comment on fungal infections.

- Fungal infections are extremely common and some of them are serious and fatal
- With the control of most bacterial infections in developed countries, incidence of fungal infections is increasing
- Most of the fungi are soil saprophytes and human infections are opportunistic
- Modern advances in treatment have increased incidence of fungal infections

■ What are fungi?

Fungi are eukaryotes, broadly divided into the following two groups:

1. **Yeast:** These are ovoid or ellipsoidal cells that reproduce by asexual process known as budding
 - Budding is an asexual method of reproduction of yeast. Daughter bud arises from parent bud, enlarges and separates from parent cell
2. **Mould:** These are filamentous fungi that reproduce sexually and/or asexually by forming spores

■ What is a hypha and a pseudohypha? Differentiate between the two.

- **Hypha:** It is an elongated tubular, thread-like branching filament of even width
- **Pseudohypha:** A chain of elongated cells is known as pseudohypha
- Features differentiating between hyphae and pseudohyphae are presented in Table 71.1

Table 71.1 Differences between hypha and pseudohypha

Hypha	Pseudohypha
• It is formed by apical elongation	It is formed by budding
• Cell walls are parallel to each other	Cell walls are constricted at septa
• Septa are straight and perpendicular	Septa are curved
• Septum and constriction is absent at origin of branches	Septum and constriction is present at origin of branches

■ Describe different types of hyphae with suitable illustrations.

Types of Hyphae

Aseptate and Septate Hyphae (Fig. 71.1)

When septa are present hypha is called septate hypha and when it is absent it is called aseptate.

Examples: Septate hyphae are present in *Aspergillus* and *Penicillium*, while aseptate are present in Zygomycetes.

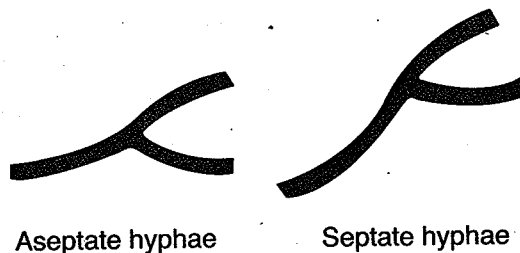


Fig. 71.1 Aseptate and septate hyphae.

Hyaline and Pigmented Hyphae

Dark coloured hypha is called pigmented and colourless is called hyaline.

Examples: Pigmented hyphae are present in phaeoid fungi such as *Wangiella*, while hyaline hyphae are present in *Aspergillus* and *Penicillium*.

Vegetative and Aerial Hyphae

- The hypha, which is submerged in the media or is on the surface of media is called vegetative hypha, while the hypha, which projects above the medium is called aerial hypha.
- Vegetative hyphae absorb nutrients from media for growth, hence concerned with nutritive function.
- Aerial hyphae carry spores, which are units of reproduction, hence concerned with reproductive function, e. g. filamentous fungi possess vegetative as well as aerial hyphae.

Depending on the shape, the following hyphae are distinguished: racquet, spiral, nodular organ, favic chandelier, pectinate (Fig. 71.2):



Spiral hyphae Racquet hyphae Favic chandelier hyphae Pectinate hyphae Nodular organ hyphae

Fig. 71.2 Different forms of hyphae, depending on their shape.

Spiral hypha

These are like coiled filaments or spirals, e.g. *Trichophyton mentagrophytes*.

Racquet hypha

Shape is like that of a racquet, one end broad and one short. Broad and short ends are in opposition, e.g. *T. mentagrophytes*.

Nodular hypha

It is swelling or enlargement in mycelium or hypha, e.g. *Microsporum canis*, *T. mentagrophytes*.

Favic chandelier

Tip of this type of hypha shows multiple short projections resembling horns of reindeer or chandelier, e.g. *T. violaceum*, *T. schoenleinii*.

Pectinate hypha

This type of hypha has short irregular projections on one side giving a broken comb appearance, e.g. *M. audouinii*.

■ What is mycelium?

- Entangled mass of hyphae is called mycelium

- In a colony it can be divided into two portions **vegetative mycelium** (mycelium in the medium concerned with nutrition) and **aerial mycelium** (mycelium projecting above the surface and concerned with reproductive function)

■ What are spores? Describe different types of spores.

Spores

Spores are the fruiting bodies produced by fungi for asexual or sexual reproduction.

Types of Spores

Asexual spores

- They are formed by budding, fission and mitosis (Fig. 71.3)
- Asexual spores are of the following types:
 1. **Chlamydo spores**—asexual spores, which are large with thick double refractile wall produced from hyphal cell. They are resistant. They store nutrients and function as spores. They may be formed in groups or occur singly. They may be terminal or intercalary
 2. **Arthrospores**—asexual spores produced as a result of fragmentation of hyphae. They are cuboidal, rectangular spores formed in chains, with a slightly thick wall and are released at maturity
 3. **Blastospores**—asexual spores produced as a result of budding
 4. **Sporangiospores**—asexual spores present inside the sporangium

Conidia

- They are asexual reproductive structures borne exogenously on specialized hyphae called conidiophores
- They are of two types —macroconidia and microconidia

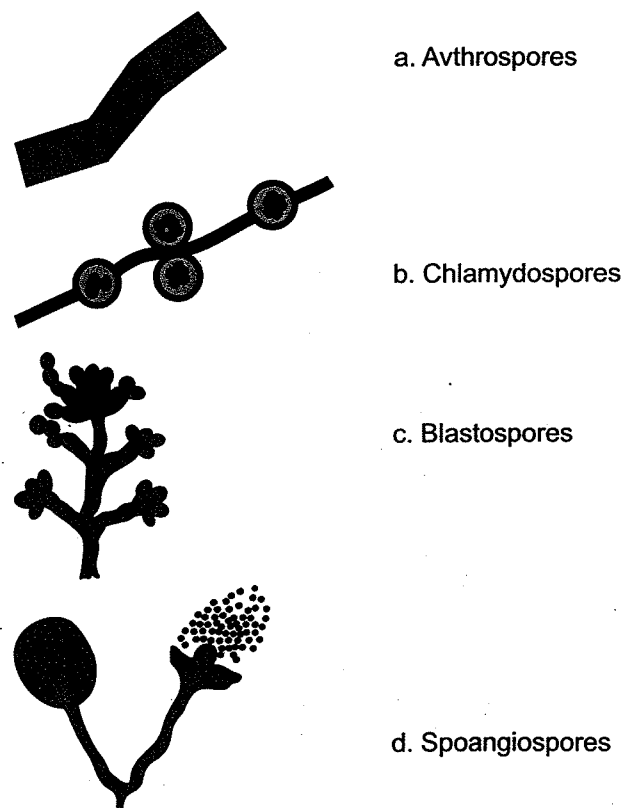


Fig. 71.3 Asexual spores.

Sexual spores

- They are produced as a result of meiosis
- Sexual spores are of the following types:
 - **Ascospores** are sexual spores inside a sac-like structure called ascus, e.g. Ascomycetes
 - **Zygosporos** are sexual spores formed by sexual conjugation of two fungi, e.g. Zygomycetes
 - **Basidiospores** are sexual spores formed on basidium, e.g. Basidiomycetes

What are the differences between fungi and bacteria?

Differentiating features of fungi and bacteria are enumerated in Table 71.2.

Table 71.2 Differences between fungi and bacteria

Fungi	Bacteria
Eukaryotes	Prokaryotes
Possess rigid cell wall containing chitin, mannan and polysaccharides	Cell wall consists of teichoic acid, muramic acid and lipopolysaccharides
Cytoplasmic membranes—contain sterols	Lack sterols
Cytoplasm consists of organelles such as endoplasmic reticulum and mitochondria	Lack mitochondria and endoplasmic reticulum
True nucleus with nuclear membrane and paired chromosomes are present	Single chromosome without nuclear membrane is present
Seen as yeast or moulds	Seen as cocci, bacilli and spirals
Reproductive spores are formed	Nonreproductive spores are formed
Most of them divide by sexual or asexual methods	Divide by binary fission

Write a note on the morphological classification of fungi.

Based on the morphology, fungi are classified into the following four groups:

1. **Yeasts:** These are unicellular fungi, which occur as spherical or ellipsoidal cells and reproduce by simple budding, e.g. *Cryptococcus neoformans* (Fig. 71.4)

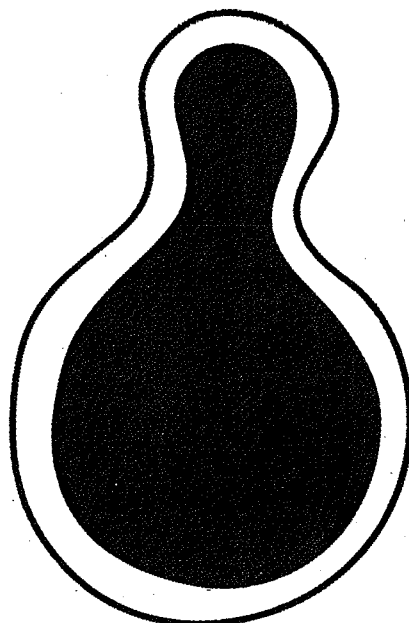


Fig. 71.4 Budding yeast cell.

2. **Yeast-like fungi:** Fungi, which reproduce by budding but the bud fails to separate, elongates and forms cells resembling hyphae known as pseudohyphae are called yeast-like fungi, e.g. *Candida albicans* (Fig. 71.5)
3. **Moulds or filamentous fungi:** Fungi, which form true hyphae and true mycelia and reproduce by the formation of different types of spores, are called moulds or filamentous fungi, e.g. Dermatophytes (Fig. 71.6)
4. **Dimorphic fungi:** Fungi, which occur in two forms, yeast as well as moulds depending on the growth conditions are called dimorphic fungi. In host tissue or in cultures at 37°C, they occur as yeast while in the soil or in cultures at 22°C, they appear as moulds or filaments, e.g. *Histoplasma capsulatum* (Fig. 71.7)

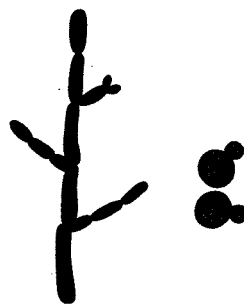
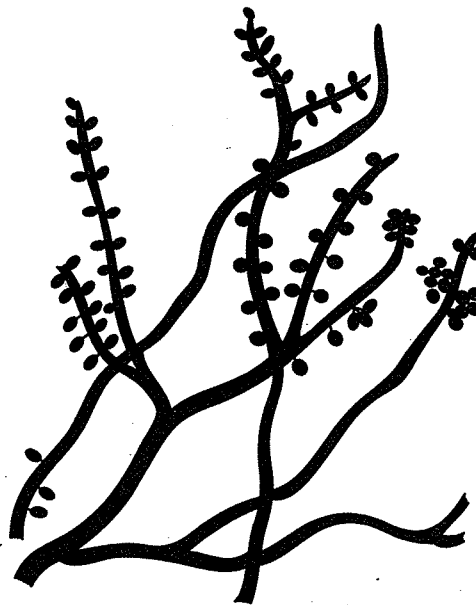


Fig. 71.5 Yeast-like fungi.



Moulds (Filamentous fungi)

Fig. 71.6 Moulds.

✓ Present the systemic classification of fungi.

Method of sexual reproduction forms the basis for the systemic classification. According to this there are four groups of fungi. These are as follows:

- **Zygomycetes**—sexual fusion forms zygospores
- **Ascomycetes**—sexual fusion forms ascus and ascospores
- **Basidiomycetes**—sexual fusion forms basidium and basidiospores
- **Fungi imperfectii**—these are fungi in which sexual process is not observed

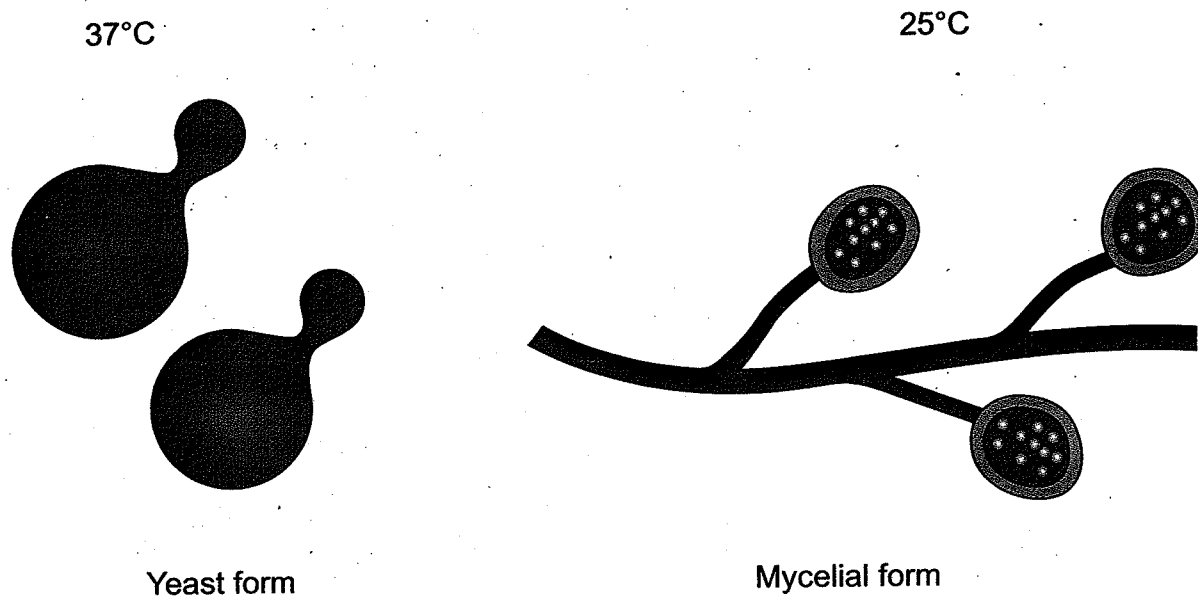


Fig. 71.7 Dimorphic fungi.

■ What factors predispose to fungal infections?

Factors predisposing to fungal infections are as follows:

- Increased use of corticosteroids
- Widespread use of antibiotics
- Use of antimalignant drugs
- Use of immunosuppressive agents
- Metabolic disorders such as diabetes mellitus
- Extremes of age – infancy and old age.

■ Describe the different types of mycoses?

Mycoses can be categorized into the following types:

1. Superficial mycoses
2. Deep mycoses
3. Opportunistic mycoses
4. Miscellaneous mycoses

LSN ■ Describe in detail the laboratory diagnosis of fungal diseases.

Specimens

Appropriate specimens are collected depending on the site of infections.

- **In superficial mycoses**—skin scraping, infected hairs and nails, mucosal scrapings or swabs
- **In subcutaneous mycoses**—pus, biopsy, discharge, crusts and swab from lesions
- **In systemic mycoses**—sputum, urine, pus, faeces, CSF, blood, biopsy, etc.

Collection and Transport of Specimen

Proper collection and transport of specimen is an important step in isolation and identification of medically important fungi.

Skin scrapings

- The affected area is cleaned with 70% alcohol
- Scrapings from the active edge of the lesion are collected in a fold of black paper with the help of scalpel blade held at right angles to skin and transported in paper fold
- Cellophane tape can also be used to collect specimen
- Specimen from scalp is collected by blunt edge of scalpel

Nail as specimen

- Discoloured and brittle part of nail is cleaned with 70% alcohol and nail pieces are collected with the help of a flame-sterilized scalpel or scissors or nail cutter
- Sample is taken from free edge and should be of full thickness
- Transported in an envelope or fold of black paper

Infected hair

- Infected hair are plucked with forceps
- Transported in an envelope or fold of black paper

Corneal scrapings

It is collected from the margin of corneal ulcer with the help of needle after giving local anaesthesia, e.g. xylocaine. The specimen is directly collected on microscopic slide and examined.

Sputum

- Early morning sputum sample is preferred
- Collected in a sterile, clean, wide-mouth, screw-capped container
- Bronchial secretions, bronchoalveolar secretions or tracheal secretions may also be collected
- Transported and processed immediately to avoid growth of commensals and to increase chances of isolation of causative agent
- Material obtained by bronchial brushing or biopsy is more suitable for diagnosis

Pus/discharge/crusts

- Aspirated with the help of syringe and needle
- Collected with the help of swab
- Crusts removed from the surface of lesion in a sterile container
- Transported immediately for further processing

Urine

- Collected by catheterization or bladder aspiration—reliable but less frequently used
- Commonly used method is “clean-catch method” to collect mid-stream urine sample
- Collected in a sterile container and transported immediately to avoid delay in processing
- If delay is unavoidable, can be refrigerated at 4°C up to 12 hours
- Urine is centrifuged and sediment is used for further processing

Cerebrospinal fluid (CSF)

- Collected in a sterile container by lumbar puncture
- 3–5 ml of CSF is collected
- Transported and processed immediately
- If delay is expected, CSF should be kept at room temperature or in an incubator

Blood

Approximately 8 ml of blood is collected aseptically by venepuncture in a brain–heart infusion broth and transported to microbiology laboratory for further processing.

Tissue biopsies

Collected by appropriate procedure in a formalin jar and transported, and processed after mincing or grinding.

Bone marrow

Collected aseptically with the help of bone marrow aspiration needle and transported like a specimen of blood, and processed.

Processing of Specimen**Direct Microscopy****1. Potassium hydroxide (KOH) mount**

- Specimens such as skin, hair, nails, sputum, etc. are examined with 1 or 2 drops of 10–20% potassium hydroxide or sodium hydroxide
- KOH dissolves keratin and other cells, and clears debris making the fungal elements more clear in 5–20 minutes depending on the nature of the specimen
- Gentle heating over the flame or addition of dimethyl sulphoxide (DMSO) hastens clearing
- Observed under low and high power objectives

2. Calcofluor white (CFW) stain

- Calcofluor white binds to polysaccharide such as glucans and chitin in cell wall nonspecifically, therefore fungal elements fluoresce brightly under fluorescent microscope

3. India ink/nigrosin wet mount

- For demonstration of capsule of *Cryptococcus neoformans*
- On a clean glass slide, a loopful of specimen is mixed with a drop of India ink/nigrosine
- A thin coverslip is placed and preparation is observed under high power objective
- Yeast surrounded by clear halo is seen against blue or black background

4. Gram stain

- For demonstration of hyphae, pseudohyphae and budding yeasts in specimens
- Fungal elements appear as Gram - positive in nature

5. Wright's or Giemsa stain

For demonstration of intracellular fungi, especially *Histoplasma capsulatum*.

6. Direct immunofluorescence stain

For demonstration of fungi in tissues and smears using antibodies conjugated with fluorescent dye.

7. Histopathological sections

The Following staining methods are used:

- (a) Periodic acid-Schiff (PAS) stain—fungal elements are stained magenta against pink-green background
- (b) Gomori's methenamine silver nitrate stain (GMS)—fungal elements are stained black against green background

Culture Methods**Media Used**

- Sabouraud's dextrose agar (SDA)
- Emmons' modification of Sabouraud's dextrose agar is used for routine cultivation of fungi

- Original Sabouraud's dextrose agar consists of 4% dextrose and 1% peptone with agar and has acidic pH, 5.4
- Emmons' modified Sabouraud's dextrose agar contains 2% dextrose and neo-peptone and has neutral pH, 7.2
- SDA with chloramphenicol (50 mg/L) and cyclohexamide (500 mg/L) helps to prevent growth of bacteria and saprobic fungi, respectively
- Media is prepared usually as slant in tubes because they are more resistant to drying
- Brain–heart infusion agar and brain–heart infusion agar with chloramphenicol and cyclohexamide can be used as alternatives
- Two slants of SDA are inoculated routinely. One of them is incubated at room temperature (25°C) and another at body temperature (37°C). Slants are incubated for 4 weeks before they are declared negative. They are observed daily for first week and twice a week thereafter. Most of the cultures become positive in 10 days

Special Media

- Czapek–Dox medium is used for *Aspergillus*
- Birdseed agar are used for *Cryptococcus*

Identification

Identification of fungi is done based on the following:

- Macroscopic appearance of colony
- Microscopic examination of colony by lactophenol cotton blue (LCB) preparation
- Slide culture
- Corn meal agar culture
- CHROM agar
- Dermatophyte identification medium
- Biochemicals such as sugar assimilation and fermentation

Macroscopic appearance of colony

The important characteristics noted are:

- Gross morphology of colony—nature and colour on obverse side of slant and pigmentation on reverse side
- Growth rate

Microscopic morphology

Lactophenol cotton blue (LCB) preparation:

Tease mount

- A small portion of colony is taken on a clean slide in a drop of lactophenol cotton blue (LCB) and teased apart with the help of dissecting needles
- This preparation is covered with a cover slip and observed under the microscope
- The morphology of fungal elements and arrangement is observed and noted
- This method has limited value as morphology of fungus may be disturbed because of teasing
- Cellophane tape can also be used with sticky side down on top of colony to remove the portion of it to drop of LCB
- The LCB is observed for morphology of
 - Hyphae—diameter, presence of septa, presence of special hyphae
 - Spores—morphology/types, arrangement

Slide culture method

- It is used to study *in situ* morphology of fungus, which helps in identification
- A block of one cubic square from SDA is taken on a sterile glass slide
- All vertical sides of the block are stab inoculated with the help of straight wire
- Block is covered with cover slip
- This preparation is then transferred to a wet chamber (Petri dish with a moistened filter paper and water)
- Incubated at room temperature
- When growth appears, a drop of LCB is added on slide after removing block and observed
- The slide culture may be observed directly under low power microscope

Corn meal agar culture

Culture on this medium helps to study sporulation of fungus, thereby helping in identification.

CHROM agar

It is a chromogenic medium that helps in presumptive identification of yeast-like fungi.

Dermatophyte identification medium

It is a chromogenic medium that helps in presumptive identification of dermatophytes.

Biochemical and other reactions

- Sugar assimilation and fermentation
- Urease test
- Germ tube formation

These tests help in identification of yeast and yeast-like fungi.

Ag Detection

For antigen detection the following tests are available for some fungi such as *Cryptococcus*, *Candida*, *Aspergillus*, etc.

- Latex agglutination test
- Enzyme-linked immunosorbent assay
- Radioimmunological assay
- Counter current immunoelectrophoresis
- Complement fixation test
- Immunofluorescence test
- Exoantigen test

Serological Tests

Used for diagnosis of various systemic fungal infections. These include:

- **Complement fixation test**—for blastomycosis, coccidioidomycosis, histoplasmosis, paracoccidioidomycosis, etc.
- **Agar gel diffusion**—for aspergillosis, blastomycosis, candidiasis, coccidioidomycosis, paracoccidioidomycosis, histoplasmosis, etc.
- **Indirect immunofluorescence test**—for cryptococcosis
- **Latex agglutination test**—for candidiasis, cryptococcosis, sporotrichosis, etc.

Nucleic Acid Detection

- The technique uses probes and polymerase chain reaction (PCR)

- Genetic probes are available for identification of some fungi, e.g.
 - *Blastomyces dermatitidis*
 - *Coccidioides immitis*
 - *Cryptococcus neoformans*
 - *Histoplasma capsulatum*

Skin Test

Commonly used in earlier days, it is not useful for diagnosis but used for epidemiological purpose

Animal Inoculation

Animal inoculation test is used

- To determine pathogenicity, or
- To demonstrate yeast form of fungus in dimorphic fungi causing systemic infections (yeast form is seen at body temperature)
- Animals used are mice, rats, guinea pigs, rabbits, hamsters, etc.

Antifungal Susceptibility Testing

Antifungal susceptibility testing is performed by

- Disc diffusion method
- Broth dilution method

■ Name different types of antifungal agents.

Different types of antifungal agents:

- **Antifungal antibiotics**
 - Amphotericin B,
 - Nystatin and
 - Griseofulvin
- **Synthetic antifungal agents**
 - Tolnaftate,
 - Imidazoles such as clotrimazole, ketoconazole and
 - Triazoles such as fluconazole, itraconazole
- **Miscellaneous antifungal agents**
 - Flucytosine,
 - Potassium iodide,
 - Selenium sulphide and
 - Gentian violet

72

Chapter

Superficial Mycoses

- **What is superficial mycoses? Write a note on superficial fungal infections and their causative agents.**

The superficial or cutaneous mycoses refers to fungal infections of the outer layers of skin, hair, and nails. Superficial mycoses is divided into the following two types:

1. Surface Infections

Infections of outer most epithelial tissue. Fungus lives on dead layers of skin producing cosmetic effects only. These fungi do not elicit allergic response because they are not in contact with living tissue, e.g. tinea versicolor, tinea nigra and piedra (Table 72.1).

2. Cutaneous Infections

These are the infections of cornified layer of the skin and its appendages. These infections are caused by dermatophytes, which elicit allergic and inflammatory response (Table 72.1). *Candida* can also cause cutaneous infections.

Table 72.1 Superficial fungal infections and their causative agents

Condition	Agent
1. Pityriasis (Tinea) versicolor	<i>Malassezia furfur</i>
2. Tinea nigra	<i>Hortaea werneckii</i>
3. Piedra	
– Black piedra	<i>Piedraia hortae</i>
– White piedra	<i>Trichosporon beigeli</i>
4. Dermatophytoses	Dermatophytes
	– <i>Trichophyton</i> ,
	– <i>Microsporum</i> ,
	– <i>Epidermophyton</i>

- **Write a note on Pityriasis (Tinea) versicolor.**

- *Pityriasis (Tinea) versicolor* is a mild, chronic superficial fungal infection of the stratum corneum, characterized by patchy discolouration of the skin.
- **Causal agent:** *Malassezia furfur* (*Pityrosporum orbiculare*). It is a part of normal skin flora and infection is mostly endogenous.
- Neck, trunk, face, scalp, upper limbs, shoulders are common sites. It equally affects both sexes affecting late teens.

Pathogenesis

Fungus interferes with melanin production.

Clinical Features

Many, well-defined, noninflammatory, macular lesions with fine scaling, discrete hypo- or hyper-pigmented patches depending on pigmentation of the surrounding skin.

Laboratory Diagnosis

Wood's Lamp Examination of Lesion

- It is used in the diagnosis of superficial fungal infections
- When lesion is examined with a lamp, it shows golden yellow fluorescence

Specimen

Skin scrapings, collected with the help of sterile scalpel transported in a fold of black paper.

Processing of Specimen

Microscopic Examination (Fig. 72.1)

- **10% KOH preparation**—shows clusters of round yeast cells, 2–7 μ m size and plenty of short, stout, curved hyphae that give banana- and grapes-like appearance or Spaghetti- and meatballs-like appearance

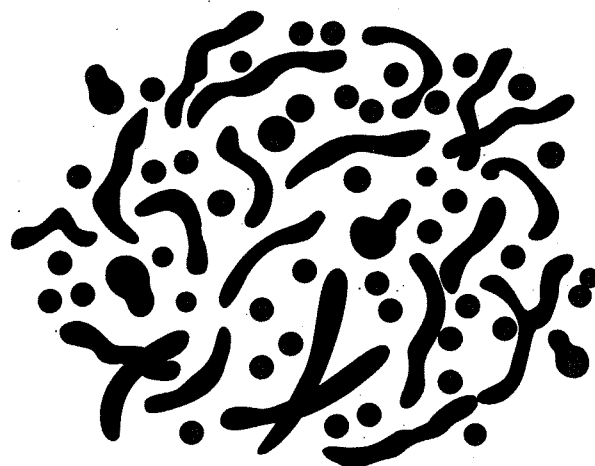


Fig. 72.1 *Malassezia furfur*.

Culture

- **Media:** SDA with antibiotics, Tween 80 and film of olive oil
- **Incubation:** 5–7 days at 30°C
- **Colony:** Small, cream to yellow
- **LCB:** Yeasts 2–7 μ m with many small bottle-shaped cells 2–3 μ m; hyphae—rarely seen

Serological Tests

- **ELISA**—to detect Ab response
- **Immunofluorescence test**—green or orange fluorescence

Treatment

Sodium thiosulphate (20%), Whitfield ointment and imidazole cream.

■ Write a note on *Tinea nigra*.

Tinea nigra is a superficial fungal infection of skin caused by a dematiaceous fungus *Hortaea werneckii*, characterized by formation of black-brown macular lesions

Thickly keratinized site such as palm and soles are commonly affected. It is common in females and in the age group under 20 years.

Clinical Features

- Asymptomatic, well-defined brown-black pigmented macular lesions enlarge slowly
- No scaling, 1–5 cm

Laboratory Diagnosis

Sample

Skin scrapings collected with the help of sterile scalpel, in a fold of black paper.

Microscopic Examination

KOH preparation—Shows brown, septate, branching hyphae 5–6 μ and budding cells 2–8 μ in diameter.

Culture

- **Media:** SDA with antibiotic and actidione
- **Colony:** Moist, yeast-like white to dark-colored colonies
- **LCB:** Brown, budding yeast-like cells with septa and dark hyphae

Treatment

Whitfield ointment, miconazole (topical).

■ **Write a note on *Piedra*.**

- *Piedra* is a superficial fungal infection of hairs characterized by formation of nodules along hair shaft
- Two types - black and white *piedra*
- Black *piedra*—*Piedraia hortae*
- White *piedra*—*Trichosporon beigelii*

Clinical Features

- Black *piedra*—black, hard, nodules along hair shaft. Scalp, moustache and beard area – commonly affected
- White *piedra*—grayish-white, soft nodules on hair shaft. Axillary, pubic, beard, moustache area – commonly affected

Laboratory Diagnosis**Specimen**

Hair pieces collected with the help of scissors in a paper envelope.

Microscopic Examination

- **KOH preparation**
 - Black *piedra*—dark septate hyphae around the surface of the hair with asci containing ascospores
 - White *piedra*—hyaline septate hyphae and arthrospores within and round shaft

Culture

- **Media:** Sabouraud's dextrose agar with antibiotics
- **Colony:**
 - Black *piedra*—brown, black, velvety heaped up centre and flat periphery
 - White *piedra*—cream coloured, yeast-like, later on develop furrow and become folded
- **LCB:** Black *piedra*—dark hyphae. White *piedra*—hyaline hyphae, blastospores and arthrospores

Treatment

Shaving of affected area, topical azole cream and oral ketoconazole.

Define and classify dermatophytes.

Dermatophytes are a group of fungi that infect only superficial keratinous tissues (skin, nail and hair).

Aetiology

Three genera namely

1. *Trichophyton*
 - infecting hair, skin and nail

2. *Microsporum*
 - infecting hair and skin
3. *Epidermophyton*
 - infecting skin and nail

Classification

1. Depending on Ecological Reservoir they are classified as follows

- (a) **Geophilic**— found in soil, e.g. *M. gypseum* and *M. nanum*
- (b) **Anthropophilic**— found in humans, e.g. *T. rubrum*, *T. mentagrophytes*, *T. violaceum*, *T. tonsuran*, *T. schoenleinii*, *E. floccosum*, *M. audouinii*
- (c) **Zoophilic**—found in animals, e.g. *T. equinum*, *T. verrucosum* and *M. equinum*

2. Depending on Morphology and Number of Macro and Microconidia (Fig. 72.2)

- (a) *Trichophyton*
- (b) *Microsporum*
- (c) *Epidermophyton*

The morphological differentiating features of macro and microconidia of the three genera of dermatophytes are highlighted in Table 72.2.

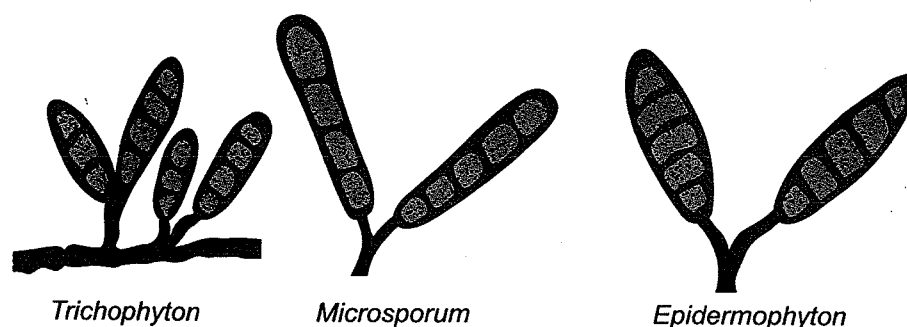


Fig. 72.2 Morphology of macroconidia.

Table 72.2 Differences between macroconidia and microconidia

Macroconidia	Microconidia
<i>Trichophyton</i>	
1. Pencil shaped, elongated, thin walled smooth	Oval or spherical, singly or in groups
2. Rarely seen	Abundant in numbers
<i>Microsporum</i>	
1. Spindle shaped with tapering ends, thick, rough walled	Rarely seen
2. Plenty in number	
<i>Epidermophyton</i>	
1. Club shaped, smooth walled in clusters	Not seen
2. Plenty in number	

✓ Briefly describe pathogenesis of dermatophytoses.

- Dermatophytes grow only in dead keratinized tissue
- Fungal cell produces keratinolytic proteases, which help in entry into living cells
- Fungal metabolites diffuse into deeper layers of skin causing erythema, vesicles, and pustules along with pruritus.

Describe in detail the clinical features of dermatophytoses.

Dermatophytes are the cause of dermatophytoses, which may clinically present as:

1. Tinea corporis

- It is infection of nonhairy skin of the body
- It shows single or multiple or confluent erythematous, scaly, annular, sharply margined lesions
- It is commonly caused by *T. rubrum* and *T. mentagrophytes*

2. Tinea cruris

- It is infection of groin, found mostly in males
- It presents as erythematous patches, scaling and itching
- It is commonly caused by *T. rubrum* and *Epidermophyton floccosum*

3. Tinea faciei

- It involves skin of face excluding beard area and present as *Tinea corporis*

4. Tinea barbae

- It is infection of beard and moustaches area of face with involvement of hair
- It presents as erythematous patches with scaling, fragile, lusterless hair and folliculitis
- It is commonly caused by *T. verrucosum* and *T. mentagrophytes*

5. Tinea manuum

- It is infection of skin of palms causing hyperkeratosis of palm and fingers
- It is commonly caused by *T. mentagrophytes*, *T. rubrum* and *E. floccosum*

6. Tinea pedis

- It is infection of plantar aspects of foot, toes and interdigital webs
- It is seen in shoe wearers
- Scaling, fissuring and maceration associated with itching and burning are seen
- The disease also called **athletes foot** and **sandal ring worm**

7. Tinea imbricata

- It is an unusual form of Tinea corporis caused by *T. concentricum*
- Concentric rings of scaling, which spread out to periphery are associated with pruritus

8. Tinea capitis

- It is infection of shaft of scalp hair and may present as inflammatory or noninflammatory lesion
- Different types are keroin, favus, ectothrix or endothrix
 - Infected hair are dull-gray, which break to form patches of alopecia
 - **Keroin:** It is painful, inflammatory lesion; form mass on scalp, which may suppurate, form sinus and discharge pus; commonly caused by *T. verrucosum* and *T. mentagrophytes*
 - **Favus:** In this condition waxy honeycomb-like crusts are formed around hair follicles and can cause patchy alopecia and scarring; commonly caused by *T. schoenleinii*, rarely by *T. violaceum* and *M. gypseum*
 - **Ectothrix:** Arthrospores form sheath or chains of arthrospores around hair shaft; commonly caused by *T. verrucosum*, *T. mentagrophytes*, *T. rubrum* and *M. gypseum*
 - **Endothrix:** Arthrospores are seen within shaft, cuticle of hair are destroyed; commonly caused by *T. schoenleinii* and *T. violaceum*

9. Tinea unguium

It is infection of nail plates, starts under free edge or laterally and involves whole plate. Nail becomes thick, chalky and opaque

10. Id Reaction

- Secondary eruptions occurring in sensitized patients because of circulation of allergic products from primary site of infection and its absorption leading to itching
- This is found in patients with absence of delayed hypersensitivity reaction to dermatophyte antigen
- **Wood's lamp examination:** In Wood's lamp examination of lesion, hair infection caused by *Microsporum audouinii*, *M. canis* and *M. ferrugineum* shows bright green fluorescence and that with *T. schoenleinii* and *M. gypseum* shows dull-green fluorescence.

✓ Describe the methods of diagnosing dermatophytoses in a laboratory.

Specimens

Skin scrapings collected with the help of sterile scalpel, hairpieces by plucking or cutting and nail pieces by nail cutter and transported in a fold of black paper

Microscopic Examination

- KOH mount
- Fungus is seen as branching, hyaline mycelia and as arthrospores
- Ectothrix and endothrix infection can also be differentiated in KOH mount (Fig. 72.3)
- Ectothrix shows chains of arthrospores inside and outside the hair shaft while endothrix shows chains inside the shaft

Culture

- **SDA with cycloheximide and chloramphenicol** is used for routine cultivation of fungi
 - Two slants are inoculated—one incubated at 25°C and another at 37°C
 - Spot inoculation is preferred on slants to differentiate growth from contaminants
 - Slants observed daily for first week and on alternate days thereafter for growth
 - Rate and nature of growth and LCB picture help in identification of the agent (Fig. 72.4, Table 72.3)
- **Dermatophyte test medium** can also be used when SDA is not available
 - It is used to isolate and differentiate dermatophytes from bacterial contaminants
 - Dermatophytes turn the medium red by raising pH of medium through metabolic activity, while most bacteria and other fungi do not
- **Malt extract agar** is used to study sporulation of fungi
- **Slide culture** can also be used to see *in situ* morphology of fungus

Identification Tests for Dermatophytes

- **Hair perforation test:** It is used to differentiate *T. mentagrophytes* and *T. rubrum* and also *M. canis* and *M. equinum*. It is positive in *T. mentagrophytes* and *M. canis*
- **Urease test:** It differentiates *T. rubrum* and *T. mentagrophytes*

Skin Test

Trichophytin skin test: The test uses trichophytin antigen. A delayed hypersensitivity-like response is seen. Patients without delayed hypersensitivity reaction are more susceptible to chronic infections.

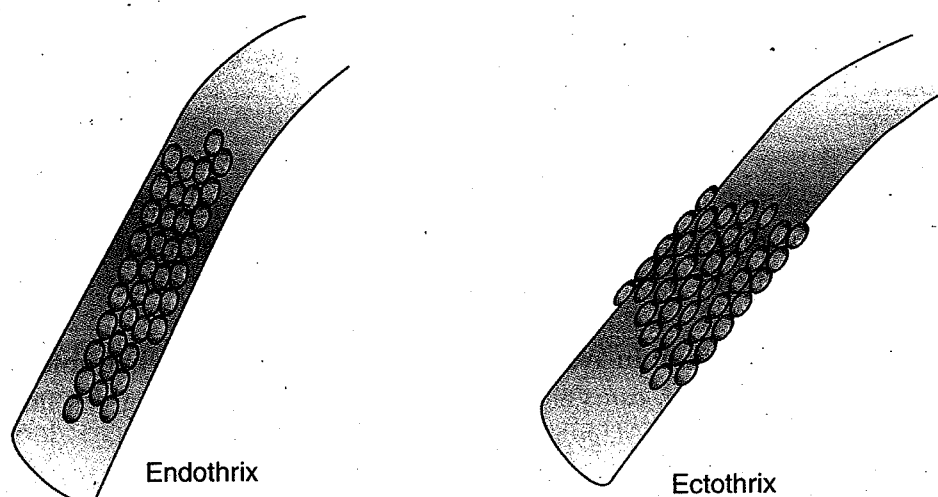


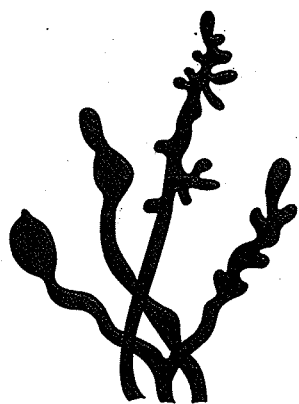
Fig. 72.3 Endothrix and Ectothrix. (Source: *Mikrobiologia*: Urban and Partner, Figure 71-12, PP. 689–698.)

Ab Detection

Immunodiffusion: It is used to demonstrate antibody response to infection.

■ **How can dermatophytoses be treated?**

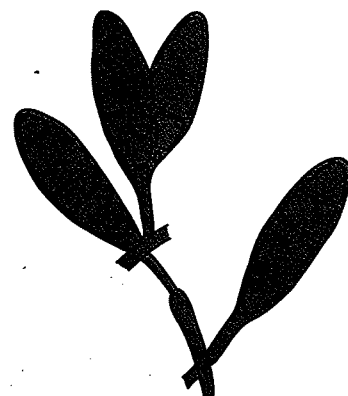
Oral griseofulvin, ketoconazole and itraconazole can be used for treating dermatophytoses



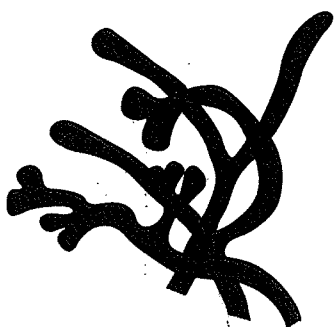
M. audouinii



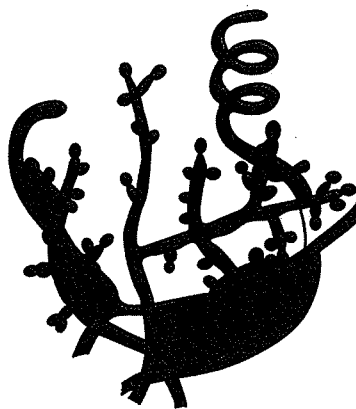
M. canis



M. gypsum



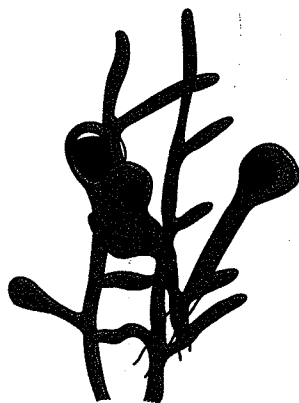
T. schoenleinii



T. mentagrophytes



T. rubrum



T. verrucosum



T. violaceum



E. floccoum

Fig. 72.4 LCB picture of various dermatophytes.

Table 72.3 Colony and LCB picture of common dermatophytes

Agent	Growth rate (Weeks)	Colony	LCB picture
<i>T. mentagrophytes</i>	2	O—Buff to pink, flat fluffy R—Buff to brown	Macroconidia—may be seen Microconidia—many spiral hyphae
<i>T. rubrum</i>	1	O—White cottony R—Red	Macroconidia—may be seen Microconidia—many
<i>T. schoenleinii</i>	1–2	O—Gray brown, folded R—Gray brown	Macroconidia—not seen Microconidia—not seen Antler hyphae (Favic chandeliers)
<i>T. verrucosum</i>	2–3	O—Buff, folded R—White or buff	Macroconidia—rare Microconidia—rare Many chlamydospores
<i>T. violaceum</i>	1–2	O—Purple, waxy R—Deep purple	Macroconidia—not seen Microconidia—not seen Chlamydospores and thick hyphae
<i>M. canis</i>	1–2	O—White, silky R—Deep yellow	Macroconidia—many with septa Microconidia—small and few Racquet hyphae
<i>M. gypseum</i>	1	O—Pale brown powdery R—Buff to yellow brown	Macroconidia—many Microconidia—few or absent
<i>M. audouinii</i>	1–2	O—Buff pale orange R—Pale orange	Macroconidia—not seen Microconidia—not seen Pectinate and thick hyphae Chlamydospores
<i>E. floccosum</i>	1	O—Gray yellow folded R—Yellow brown	Macroconidia—present Microconidia—not seen Many chlamydospores Distorted thick hyphae

LCB = lactophenol cotton blue, O = obverse, R = reverse.

73

Chapter

Deep Mycoses

■ Mention different types of deep mycotic infections.

Deep mycotic infections include:

- **Subcutaneous mycoses**—Mycoses of subcutaneous tissues, usually follows trauma
- **Systemic mycoses**—Mycoses of internal organs primarily affecting lungs. Opportunistic fungi can also cause systemic infections

■ Mention the diseases and causal agents of different types of subcutaneous mycoses.

The disease conditions and their associated fungi are presented in Table 73.1.

Table 73.1 Subcutaneous mycoses

Condition	Causal agent
Mycotic mycetoma	<i>Madurella mycetomatis</i> <i>M. grisea</i> <i>Pseudallescheria boydii</i>
Rhinosporidiosis	<i>Rhinosporidium seeberi</i>
Sporotrichosis	<i>Sporothrix schenckii</i>
Chromoblastomycosis	<i>Fonsecaea</i> spp. <i>Phialophora verrucosa</i> <i>Cladophialophora carrionii</i> <i>Rhinocladiella aquaspersa</i>
Phaeohyphomycosis	<i>Bipolaris</i> , <i>Curvularia</i> , <i>Alternaria</i> <i>Exophiala</i> , <i>Phialophora</i> , <i>Wangiella</i> <i>Cladophialophora</i>
Lobomycosis	<i>Lacazia loboi</i>

▼ What is mycetoma?

Mycetoma is a subcutaneous fungal infection, usually involving extremities, caused by bacteria as well as fungi and characterized by clinical triad of tumefaction, sinus formation and discharge of granules. Its first case was reported from Madurai district South India, hence it is called **madura foot** or **maduramycosis**.

■ Classify mycetoma on the basis of causative agents and colour of granules.

Classification of Mycetoma

- **Based on causative agents**

It is of the following two types:

1. **Actinomycetoma**—mycetoma caused by bacteria
2. **Eumycetoma**—mycetoma caused by fungi

- **Based on colour of granules**

It can be classified as follows:

1. Black grain mycetoma
2. White grain mycetoma

✓ **List the bacterial and fungal agents responsible for causing mycetoma.**

Agents responsible for causing mycetoma are as follows:

Bacterial Agents

1. *Actinomadura madurae*
2. *A. pelletieri*
3. *Nocardia brasiliensis*
4. *N. caviae*
5. *N. asteroides*
6. *Streptomyces somaliensis*

Fungal Agents

1. *Madurella mycetomatis*
2. *M. grisea*
3. *Exophiala jeanselmei*
4. *Curvularia geniculata*
5. *Pseudallescheria boydii*
6. *Acremonium* spp.
7. *Fusarium* spp.
8. *Aspergillus nidulans*

■ **Comment on geographical distribution of mycetoma.**

- Mycetoma occurs in tropical and subtropical countries of Asia, especially the developing countries
- In India, eumycetoma is more prevalent in North India as compared to South India and actinomycetoma is more prevalent in South India
- More number of mycetoma cases are seen in Tamil Nadu and Rajasthan in comparison to other states

■ **Which age group and sex are most likely to acquire mycetoma and why?**

- Majority of cases are found in the age group of 20–40 years - an active age group commonly involved in outdoor activity, in which chances of trauma are more. Males are more commonly affected with mycetoma than the females.

■ **How is mycetoma transmitted to human beings?**

Mycetoma causing organisms are found in soil and transmitted through subcutaneous inoculation of causal agent by trauma or thorn prick.

✓ **What are the clinical features of mycetoma?**

- Common sites affected by mycetoma are extremities, back, head, shoulder and buttocks, but may involve any site.
- Introduction of agent by trauma leads to localized painless swelling, which forms discharging sinuses. Colour, size and shape of granules depend on aetiological agent. Infection may involve deeper sites
- Clinically, eumycetoma shows some differences from actinomycetoma (Table 73.2)

Table 73.2 Differences between eumycetoma and actinomycetoma

Features	Eumycetoma	Actinomycetoma
1. Agents	Fungi	Bacteria
2. Extent of involvement	Less extensive	More extensive
3. Swelling	Single with well-defined margins	Multiple diffuse with ill-defined margins
4. Colour of granules	Black or white	White with exception of <i>A. pelletieri</i>
5. Discharge	Serous	Purulent

Describe the laboratory diagnosis of mycetoma.

Specimens

- Pus, exudates, grains and biopsy of affected tissue.

Collection and Transport

- Clean the sinus and press from periphery to collect samples
- Pus, exudates are collected in a sterile test tube
- Granules may be collected by using gauze piece, probe or loop
- Samples are collected in a test tube containing normal saline and allowed to stand at room temperature

Processing of Specimen

Gross Examination of Granules

Sedimented granules are observed for colour, size and texture

Colour

- **Black granules**—are found in mycetoma caused by
 - *Madurella mycetomatis*
 - *M. grisea*
 - *Exophiala jeanselmei*
 - *Curvularia geniculata*
- **White granules**—are found in mycetoma caused by fungi such as
 - *Pseudallescheria boydii*
 - *Acremonium* spp.
 - *Fusarium* spp.
 - *Aspergillus nidulans* and bacteria except *A. pelletieri*
- **Pink-red granules**—are produced by *A. pelletieri*

Size

Usually size is below or equal to 1 mm—in mycetoma caused by all except the following two:

- 2 mm in mycetoma caused by *Aspergillus nidulans* and *A. madurae*
- 5 mm in mycetoma caused by *Madurella mycetomatis*

Texture

- Hard in mycetoma, caused by *M. mycetomatis*, *Curvularia geniculata*, *Streptomyces* and *A. pelletieri*
- Soft in mycetoma, caused by all other agents

Microscopic Examination

Crushed granules are examined by the following methods:

- **KOH preparation:** This method is used to find out hyphae and other fungal elements; their presence indicates fungal aetiology
- **Gram stain:** Smear of granules stained by Gram stain is examined for presence of Gram-positive filamentous bacteria
- **Acid fast stain using 1% sulphuric acid:** Smear of granules stained by acid fast stain shows acid fast filamentous bacteria suggestive of *Nocardia*

Culture

- **Media:** Sabouraud's dextrose agar, brain-heart infusion agar and Lowenstein-Jensen media
- Culture is inoculated in duplicate set of media and incubated at 37°C and 25°C
- Colony characters and lactophenol cotton blue picture of agents are used for identification of causative agents

Serological Tests

Serological tests are not useful in diagnosis.

■ Mention the treatment options for eumycetoma and actinomycetoma.

- Eumycetoma can be treated with—oral ketoconazole or itraconazole, radical surgery
- Actinomycetoma can be treated with—antibiotics such as tetracycline, streptomycin, amikacin

■ Write short note on rhinosporidiosis.

- Rhinosporidiosis is a subcutaneous fungal infection caused by the fungus *Rhinosporidium seeberi* and characterized by the formation of polyps, usually in nose.
- *R. seeberi* shows following developmental forms
 1. **Trophocyte** - Found in connective tissue as rounded oval spore, 6–8 μm in size
 2. **Sporangia** - Trophocyte gradually increases in size to form a sporangium of size 200 μm , it has thick wall about 3–5 μm and two layers—outer thick chitinous wall and inner thin membranous cellulose layer.
 3. **Endospores** - Present in sporangia - about 16,000 endospores are present which are released by rupture of sporangium or through pore one by one. After release they enter the surrounding tissue or carried by lymphatic currents
- Rhinosporidiosis is found in Asia, especially India and Sri Lanka. In India, it is common in Tamilnadu, Kerala and some other states of South India. It is common in people in the age group of 20–40 years and Males are more affected than females.
- It is common in people who take bath in stagnant water along with domestic animals.

Clinical forms

- Clinically, rhinosporidiosis may present as one of the following forms depending on the site involved: Nose, eyes, cutaneous surface near face and genitals are affected
- **Nasal rhinosporidiosis**
 - It is the commonest site involved in rhinosporidiosis.
 - It presents as a friable, bright pink coloured, highly vascular, polypoid mass in nose, giving cauliflower-like appearance.
 - It bleeds easily on touching.
 - Clinically, the patient may present as epistaxis, nasal obstruction and foreign body sensation in nose

- **Ocular rhinosporidiosis**

- It is a site involved next to nose in frequency. Lesion is usually small, fleshy, flat or pedunculated, pink, accommodating in eye. Clinically, the patient presents as foreign body sensation, increased lacrimation, photophobia, blood-stained discharge

- **Cutaneous rhinosporidiosis**

- Wart-like sessile mass in area adjoining face and nose is observed. Usually presents as subcutaneous nodules, which may ulcerate

- **Miscellaneous rhinosporidiosis**

- It may involve urethra, vagina, vulva, rectum and penis. It presents as papillomatous lesion, but it is not a sexually transmitted disease

Laboratory diagnosis.

Specimens

- Nasal washings, biopsy of the affected tissue. Nasal washing is collected by pushing few ml of saline and aspirated it back in sterile containers and processed.

Microscopic Examination

- Histopathological sections stained with H and E, PAS show hyperplastic epidermis, oedematous connective tissue and sporangium with hyaline wall, filled with endospores
- Sporangium is 200–300 μm in diameter, and wall is 5 μm thick in younger stage and becomes thin at maturity to form pore (Fig. 73.1).

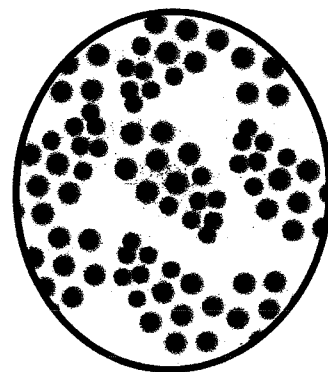


Fig. 73.1 *Rhinosporidium*—sporangium with endospores.

Culture

It cannot be cultivated on Sabouraud's dextrose agar. It can be cultivated on epithelial tissue culture.

Treatment for Rhinosporidiosis

Radical surgery is first choice. Dapsone is used for treating recurrences

■ Write a note on sporotrichosis.

- Sporotrichosis is a chronic granulomatous fungal infection of skin and subcutaneous tissue caused by *Sporothrix schenckii* – a dimorphic fungus. Disease is also known as Rose gardener's disease.
- Sporotrichosis is common in USA and South Africa
- In India, it is more prevalent in northern states such as Himachal Pradesh to Assam
- It is also reported from southwestern states of Maharashtra and northern Karnataka, particularly in alcoholics and immunocompromised persons
- *Sporothrix schenckii* is more common in males and young people due to their frequent contact with sources.
- Gardeners, carpenters, florists, mine workers are at risk of acquiring sporotrichosis.

Clinical features

Sporothrix schenckii enters the body through skin by inoculation due to trauma affecting extremities, face and neck.

Sporotrichosis occurs in the following clinical forms:

- **Lymphocutaneous sporotrichosis**

- It manifests as small, firm nodule, which becomes violaceous, may ulcerate, enlargement of lymph nodes and indurated cord-like lymph channels

- **Mucocutaneous sporotrichosis**
 - Erythematous, ulcerative and suppurative lesions usually in mouth, pharynx and vocal cord, associated with pain and enlarged lymph nodes
- **Fixed cutaneous sporotrichosis**
 - Localized to inoculation site. It forms papular ulcerative lesion, which does not spread and is usually on face, neck and trunk
- **Disseminated sporotrichosis**
 - A rare variety found in immunocompromised hosts
- **Pulmonary sporotrichosis**
 - A rare form occurring due to inhalation of conidia and manifests as chronic pneumonitis or sporotrichoma in lungs

Laboratory diagnosis

Specimens

- Exudates, pus, aspirate and swab from lesion.

Microscopic Examination

- **KOH:** Found in less numbers in specimens, hence microscopy is less sensitive for the diagnosis
- **Calcofluor white:** Its use helps to increase sensitivity
- **Histopathological examination (H and E, PAS):**
- The characteristic feature of the fungus is the **asteroid body** – round or oval yeast-like body, 3–5 μm in diameter, basophilic in nature with rays of an eosinophilic substance radiating from yeast cells.

Culture

- Sabouraud's dextrose agar, brain-heart infusion agar
- Two media are inoculated and incubated one at 25°C and another at 37°C
 - **Colony on media at 25°C**—Off-white or cream, turn velvety brown-black after 3–5 days
 - **LCB**—shows thin delicate hyphae (1–2 μm in diameter) and carry flower-like clusters of conidia borne on delicate sterigmata (Fig. 73.2)
 - **Colony on media at 37°C**—Round, oval, yeast-like colony
 - **LCB**—Budding yeast cells - Cigar-shaped yeast cells

Ag Detection – by direct fluorescent antibody technique.

Ab Detection – by Latex agglutination, Immunodiffusion and Complement fixation test. They are used for diagnosis and prognosis of patient and positive in extracutaneous form.

Skin Test

Sporotrichin test—useful for epidemiological survey.

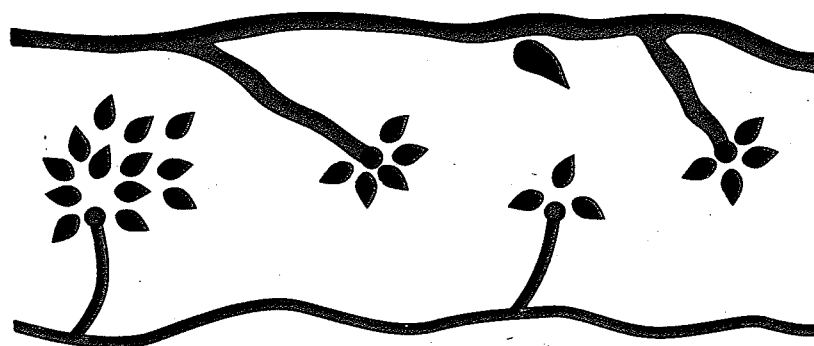


Fig. 73.2 *Sporothrix schenckii*. Group of pyriform spores are attached to conidiophore.

Animal Pathogenicity

Mice or rats are used; after inoculation they develop severe orchitis and testes show cigar-shaped cells.

Treatment

Cutaneous form of sporotrichosis can be treated with—saturated solution of potassium iodide and systemic form with—itraconazole and ketoconazole.

■ **Write a short note on chromoblastomycosis.**

- Chromoblastomycosis is a slowly progressing, localized infection of skin and subcutaneous tissue, caused by a group of dematiaceous fungi and characterized by formation of sclerotic bodies in lesion.
- It usually involves exposed parts and forms raised crusted lesions. It is also called **chromomycosis**.
- Chromoblastomycosis is caused by the following fungi:
 1. *Fonsecaea pedrosoi*
 2. *Phialophora verrucosa*
 3. *Cladophialophora carrionii*
 4. *Fonsecaea compacta*
 5. *Rhinocladiella aquaspersa*
- Chromoblastomycosis is more common in males, transmitted through inoculation (through trauma) walking barefooted and using minimal clothing are the predisposing factors. Legs, hands, shoulders, arms and face are the commonly involved sites.

Clinical features

- Presents as localized infection which does not disseminate. Initially solitary lesion is formed, which may show slow, localized spread
- Nodular, verrucose, tumoral plaque-like lesion is seen
- Verrucose lesions are ulcerated, raised above the surface giving a cauliflower-like appearance

Laboratory diagnosis

Specimens

- Dry crusty material from lesion collected with the help of sterile forceps and transported in sterile container.

Microscopic Examination

- **KOH preparation:** Round, dark brown, yeast-like body with septa called sclerotic body is a characteristic of tissue form of all causative agents
- **Histopathological section stained with H and E:** Shows sclerotic bodies

Culture and Colony

- Cultured on SDA with antibiotics and cycloheximide
- Colony—floccose gray-black with black reverse. It is difficult to identify fungi from colony characters
- Type of sporulation helps in their identification.

Treatment - treated by the following methods:

- Cryotherapy
- Laser therapy
- Surgery

- Thermotherapy
- Antifungal agents are not satisfactory but are used in extensive lesions

■ Write a short note on phaeohyphomycosis.

- Phaeohyphomycosis is a subcutaneous fungal infection caused by a group of dematiaceous fungi, characterized by presence of hyphal tissue form.
- Aetiology – caused by *Bipolaris*, *Curvularia*, *Alternaria*, *Exophiala*, *Phialophora*, *Wangiella* and *Cladophialophora*
- Found in soil, decaying vegetation and enter usually through skin.

Clinical features

Clinically, phaeohyphomycosis presents in the following forms:

- **Cutaneous phaeohyphomycosis:** Colonize skin over cracked, fissured area of sole and feet
- **Subcutaneous phaeohyphomycosis:** Presents as localized infection in the form of cyst, nodule, small subcutaneous palpable masses
- **Invasive and cerebral phaeohyphomycosis:** A serious form of phaeohyphomycosis, occurring due to haematogenous spread, specially in immunocompromised patients due to some underlying conditions.
- **Paranasal sinus phaeohyphomycosis:** Confined to paranasal sinuses

Laboratory diagnosis

- Specimens- Aspirate from cyst, curetting from nodule.

Microscopic Examination

- KOH shows pigmented dark brown hyphae
 - Hyphae vary in size and shape and are septate
 - Swollen hyphae may also be seen
 - Yeast-like cells, single or in short chains and spherical cells like chlamydospore are also seen
- Fontana-Masson stain shows presence of melanin in cell wall and septa of hyphae of phaeoid fungi

Culture

- **Media:** SDA with cycloheximide
- **Colony:** It grows slowly taking 2–4 weeks and forms black yeast-like colony, which becomes mycelial later on. LCB of colony helps in identification of fungi.

Treatment

Treated by excision of fungal mass.

■ Tabulate clinical conditions of systemic mycoses along with their causative agents.

Clinical conditions of systemic mycoses are presented in Table 73.3.

✓ 5N ■ What is candidiasis? Mention its Aetiology, Source of infection, and Predisposing factors.

- Candidiasis is the commonest fungal infection in humans, causing superficial and deep infections, caused by the fungi *Candida*.

Table 73.3 Systemic mycoses

Condition	Causative agent
1. Candidiasis	<i>Candida</i> species
2. Cryptococcosis	<i>Cryptococcus neoformans</i>
3. Histoplasmosis	<i>Histoplasma capsulatum</i>
4. Blastomycosis	<i>Blastomyces dermatitidis</i>
5. Paracoccidioidomycosis	<i>Paracoccidioides braziliensis</i>
6. Coccidioidomycosis	<i>Coccidioides immitis</i>

Aetiology

Genus *Candida* includes 20 species pathogenic to humans. Medically important species are:

- *C. albicans*
- *C. tropicalis*
- *C. krusei*
- *C. guilliermondii*
- *C. glabrata*
- *C. parapsilosis*
- *C. kefyr*
- *C. viswanathii*

The commonest pathogenic species is *C. albicans*, which has two serotypes—A and B based on their differences between mannan components of the cell wall.

Source

They are part of normal flora of human beings and are commonly found on skin, gastrointestinal tract and female genital tract, hence infection is usually endogenous in origin.

Predisposing Factors

- Naturally susceptible age groups are—infancy, pregnancy, old age
- Change in local normal flora due to antibiotics
- Change in surface as moisture and trauma
- Primary and secondary immunodeficiency
- Metabolic diseases like diabetes mellitus
- Deficiency of zinc and iron.

✓ Describe the clinical forms of candidiasis.

- *Candida* can cause superficial and deep infections, which can be divided into mucocutaneous diseases, cutaneous diseases, systemic infections and allergic diseases
- Diseases caused by *Candida* are as follows:

Mucocutaneous Diseases

Oral Candidiasis

The most common candidal disease, also called **oral thrush** involves buccal mucosa, gums and palate. It starts with congestive reddening of mucosal membrane, and give dry, smooth, shiny appearance

Gastrointestinal Candidiasis

Oesophagus and stomach are mainly involved. It manifests as burning pain in epigastrium and gastritis. In oesophagoscopy it shows white mucosal plaques

Ocular Candidiasis

Long-term use of topical steroid leads to candidal keratoconjunctivitis, which is characterized by oedema, cheesy discharge and corneal ulcerations.

Vulvovaginitis

Vaginal candidiasis is common in young middle-aged females and characterized by itching, burning, curdy vaginal discharge and dyspareunia.

Balanitis, Balanoposthitis

It occurs in uncircumcised and diabetic men and characterized by erosions and oedema of prepuce.

Chronic Mucocutaneous Candidiasis

Found especially in patients with deficient CMI, particularly infancy. Multiple lesions on skin, nail and mouth are seen but there is no visceral involvement.

Cutaneous Diseases**Skin Lesions**

- **Intertrigo:** It is an inflammatory lesion of skin-fold, which may show secondary infection with lymphangitis and lymphadenopathy
- **Diaper dermatitis:** It is diaper rash in infants.
- **Granuloma:** It is vascularized papule covered with brown crust. It is found on face and scalp

Nail Lesions

- **Paronychia:** It is infection of nail-fold—it is usually at distal portion of nails
- **Onychomycosis:** It is infection of nails

Systemic Diseases**Urinary Tract Infections**

It may involve kidneys and lower urinary tract and cause candiduria.

Cardiovascular Involvement such as Endocarditis

Subacute endocarditis usually occurs in patients with abnormal prosthetic valve.

Respiratory System such as Lung Involvement

It is due to haematogenous spread leading to pneumonia in immunocompromised patients.

Central Nervous System Involvement such as Meningitis

It is observed in neonates with candidaemia especially in complicated neurosurgical patients with ventricular shunts and intracerebral prosthetic devices.

Blood Invasion

It occurs in immunocompromised patients and is caused by *C. albicans*, *C. tropicalis*, *C. parapsilosis* and *C. krusei*.

Bone and Joint Involvement

Arthritis due to haematogenous dissemination. Direct inoculation may occur during surgery and intra-articular injections. Osteomyelitis may be due to haematogenous dissemination

Eye Involvement

Endophthalmitis may occur due to disseminated infection causing blindness.

Describe the laboratory diagnosis of candidiasis.

Specimens

Appropriate samples are collected depending on site involved, e.g. swabs from local lesions, urine from UTI patients, sputum in respiratory tract infections, etc.

Microscopic Examination

- **KOH preparation:** Wet mount is prepared from sample and observed for yeasts, budding and pseudohyphae (Fig. 71.5)
- **Gram's stain:** It shows Gram-positive budding yeasts and pseudohyphae
- **Calcofluor white (CFW) stain:** Shows fluorescence if fungal elements are present
- **Histopathological sections:** The slide is stained with H and E, PAS and GMS stain and observed for yeasts, budding, pseudohyphae
 - Demonstration of pseudohyphae is important as it indicates colonization and invasion of tissue

Culture

- **Media:** SDA with antibiotics—two slants are inoculated, one incubated at room temperature and another at 37°C. Culture examined daily for first week and twice a week after that
- Corn-meal agar, tetrazolium reduction medium (TRM) and CHROM agar help in species identification

Colony characters

- **On SDA** smooth, yeasty, cream colonies are seen (Fig. 73.3)
 - LCB shows presence of yeast and pseudohyphae

Identification of species

Following tests are used for identification

- Germ tube test
- Chlamydospore production
- Biochemical tests

Germ tube test

- It is also called Reynolds–Braude phenomenon
- When portion of colony is incubated with normal human serum at 37°C for 2 to 4 hours and observed as wet preparation, it shows long tube-like projection extending from yeast cell known as germ tube
- Test is positive in *C. albicans* (Fig. 73.4)

Chlamydospore production

- Strain inoculated on corn-meal agar and incubated at room temperature for 2 to 4 days shows formation of highly refractile, thick walled, terminal spores known as chlamydospores
- It is positive in *C. albicans* (Fig. 73.5)

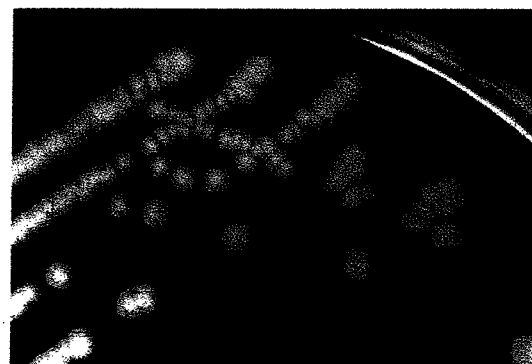


Fig. 73.3 *Candida albicans* colonies on SDA.

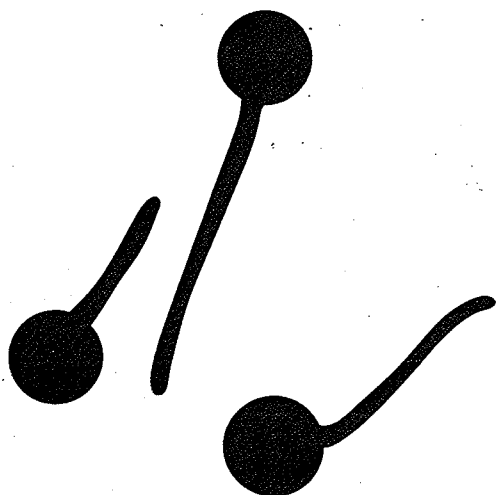


Fig. 73.4 Germ tube.



Fig. 73.5 Chlamydozoospores.

Biochemical tests

- Sugar assimilation and fermentation tests are used for identification of species
- *C. albicans* ferments glucose, maltose but not sucrose and lactose
- It assimilates sucrose, lactose, galactose, cellobiose, trehalose, glucose and maltose

Ag Detection – can be detected by

- ELISA
- RIA
- Latex agglutination
- Counter current immunoelectrophoresis
- Immunoblot

Ab Detection– can be detected by

- ELISA
- Immunofluorescence test
- Immunodiffusion
- Slide agglutination

Nucleic Acid Detection by PCR and DNA probe

Skin Test

It is not useful for diagnosis but is used to evaluate cell-mediated immunity.

Animal Pathogenicity

Mice and rabbits are used for experimental studies.

■ **Mention the drugs used in the treatment of candidiasis.**

- For cutaneous, mucocutaneous variety—1% gentian violet, nystatin, clotrimazole, ketoconazole
- For systemic variety—amphotericin B, ketoconazole, itraconazole, fluconazole

LSN ■ **What is cryptococcosis?**

- Cryptococcosis is a fungal infection caused by an encapsulated yeast belonging to genus *Cryptococcus*. It can cause infections in normal as well as immunocompromised patients.

■ Which fungus causes cryptococcosis? Name its varieties and serotypes.

- *Cryptococcus neoformans* is the most pathogenic species of the genus *Cryptococcus* to man
- It has 3 varieties and 5 serotypes
- **Varieties:** 1. *Cr. neoformans* var. *neoformans* 2. *Cr. neoformans* var. *gattii* 3. *Cr. neoformans* var. *grubii*
- These are based on morphological, biochemical and genetic characters.
- **Serotypes:** A, B, C, D, AD - These serotypes are based on capsular antigen.
- **Other species** are rarely pathogenic, e. g. *Cr. albidus* and *Cr. laurentii*

Important features:

- **Habitat**- It is ubiquitous in nature, isolated from many saprobic sources. Isolated from pigeon droppings, nests and old buildings also.
- **Route of entry**- It enters the human body mostly by inhalation; and rarely through skin.
- *Cryptococcus* is more common in males and affects people in the age group 30–50 years.

■ Describe the clinical features of cryptococcosis.

- Fifty per cent infections are found in normal individuals, 50% are in immunocompromised patients
- Cryptococcosis can be of the following forms:

1. Pulmonary Form

- Results in signs of pneumonitis. Later on it spreads to extrapulmonary sites. Clinically, it resembles any other respiratory disease characterized by chest pain, cough and fever.

2. CNS Form

- Infection of brain and meninges is a common finding in cryptococcosis.
- It manifests as meningoencephalitis or granuloma of brain
- Meningoencephalitis is characterized by meningeal signs such as neck stiffness and enlarging cryptococcoma
- It may present as intracranial space occupying lesion and presents with bilateral headache, nausea, dizziness, vomiting, impaired memory and visual disturbances

3. Visceral Form

Lung is the commonest site of infection followed by meninges, optic nerve and choroids

4. Cutaneous Form

- Infection may be caused by trauma or it may be due to haematogenous dissemination
- The scalp, face, neck may be involved. Lesions such as papules, subcutaneous nodules or lesion like molluscum contagiosum, 3–6 mm in diameter, pink, smooth with depression appear
- Cutaneous lesions due to haematogenous dissemination are common in immunodeficient persons

5. Osseous Form

Bones are involved in a few cases, characterized by swelling and tenderness for a long period

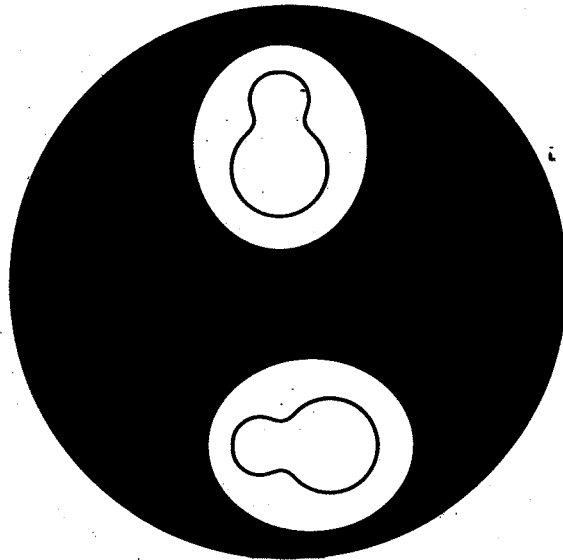
✓ Mention the methods used in laboratory diagnosis of cryptococcosis.

Specimens

CSF, sputum, exudates, etc. are collected in a sterile container using standard guidelines.

Microscopic Examination (Fig. 73.6)

- **Negative stain by using India ink or nigro-sin:** It shows round budding yeast cells ranging 5–20 μ in size with distinct halo and wide refractile capsule having diameter twice to that of central yeast
- **Gram's stain:** It shows Gram-positive budding yeast cells
- **Calcofluor white:** Used to demonstrate fungus under fluorescent microscope
- **Histopathological examination:** Section stained with H and E, PAS show budding yeast cells

**Fig. 73.6** *Cryptococcus neoformans*.**Culture**

- **Media:** SDA, blood agar, birdseed agar, niger seed agar
- Two slants of SDA are inoculated and incubated at 37°C and 25°C and observed for a period of 4 weeks
- **On SDA**—highly mucoid, cream coloured colonies are observed
- **On birdseed agar**—brown colonies due to conversion of substrate to melanin by phenol-oxidase
- Capsule production can be improved by growing fungus on chocolate agar in a CO₂ incubator at 37°C, mice inoculation and by addition of 1% peptone
- **LCB:** It shows encapsulated yeast

Biochemical Tests – such as

- Urease–positive
- Assimilation of inositol
- Assimilation of nitrate

Confirmation of species is done by

- Growth at 37°C
- Brown colonies on birdseed agar
- Mice pathogenicity
- Urease test
- Inositol assimilation
- Nitrate assimilation

Serology

To demonstrate Ag and Ab in serum, urine and CSF.

Ag Detection

Cryptolates agglutination test is used. The titre is highest in serum, intermediate in CSF and lowest in urine. A titre of 1:8 or more is significant.

Ab Detection by Agglutination, Indirect fluorescent Ab and Complement fixation test

Nucleic Acid Detection by PCR.

Animal Pathogenicity

Culture suspension is inoculated by intracerebral route in mice, animal dies in 7–10 days, or autopsy is performed to see yeast in brain tissue and body fluids.

■ Which drug is used in the treatment of cryptococcosis?

Amphotericin B, 5-fluorocytosine, miconazole, ketoconazole, itraconazole, fluconazole, etc. may be used for treating cryptococcosis.

152 ■ Define histoplasmosis. Mention its aetiology.

- It is a systemic fungal disease caused by a thermally dimorphic fungus *Histoplasma capsulatum* characterized by involvement of reticuloendothelial tissue. It is also known as Darling's disease.

Aetiology

- *Histoplasma capsulatum*
- Three medically important varieties are:
 - *H. capsulatum* var. *capsulatum*
 - *H. capsulatum* var. *duboisii*
 - *H. capsulatum* var. *farciminosum*

■ State the geographical distribution of histoplasmosis.

Histoplasmosis is distributed worldwide, mostly in temperate climate along river valley. It is endemic in US bordering Ohio and Mississippi rivers. In India, the first case was reported from Kolkata.

■ How is histoplasmosis transmitted?

Histoplasmosis is transmitted through inhalation of conidia. Man to man and animals to men transmission is not known. Males are commonly affected than females.

■ Describe the clinical features of histoplasmosis.

There are following three types of histoplasmosis:

- Histoplasmosis capsulati
- Histoplasmosis duboisii
- Histoplasmosis farciminosi

Histoplasmosis capsulati

- It is also called classical histoplasmosis
- Infection is asymptomatic in about 90% of infected persons, which can be detected by positive histoplasmin test
- Reactivation of asymptomatic infection may occur in patients with serious underlying disorders such as AIDS or organ transplantation
- It manifests as:
 - Acute pulmonary histoplasmosis
 - Chronic pulmonary histoplasmosis
 - Cutaneous and mucocutaneous histoplasmosis
 - Disseminated histoplasmosis

Acute pulmonary form

It shows symptoms like other respiratory diseases

Chronic pulmonary form

Infection may remain latent for a long time and produce features like acute pulmonary histoplasmosis but in pronounced form. It may present as apical, subapical cavity with haemoptysis.

Cutaneous and mucocutaneous form

Granulomatous and ulcerative lesions may develop on the skin and mucous membranes. In India, oral cavity involvement is common.

Disseminated form

This form is common in AIDS patients, generally presents as deterioration of health, hepatosplenomegaly and lymphadenopathy along with systemic involvement

■ Describe the laboratory diagnosis of histoplasmosis.

Specimens

- Sputum, bone marrow, peripheral blood, biopsy.

Microscopic Examination (Fig. 73.7a)

- **Sputum smear** stained with Giemsa shows yeast cells about 2–4 μm in diameter with budding, usually intracellular
- **Blood smears** from buffy coat stained with Giemsa—cell wall light blue and dark blue protoplasm with clear space in between
- **Fluorescent antibody stain**—antibody conjugated with fluorescent tags used for detection of fungus in smears

Culture

- **Media:** Sabouraud's dextrose agar, brain–heart infusion agar
- Two media inoculated and incubated—one at 25°C and another at 37°C
- Cultures are examined for 4–5 weeks before declaring negative
- **Colony on media at 25°C**—Cottony mycelial white to brown colony
 - **LCB:** It show two types of conidia—macro- and microconidia
 - Macroconidia are large, oval round, 8–14 μm in diameter, covered with finger-like projections, therefore called tuberculate macroconidia. They are diagnostic for histoplasma
 - Microconidia are elliptical, smooth-walled, 2–6 μm in diameter, borne on short hyphae, which arise at right angles to vegetative hyphae (Fig. 73.7b)
- **Colony on media at 37°C:** Cream, mucoid yeast-like colony
 - **LCB:** It shows narrow neck budding

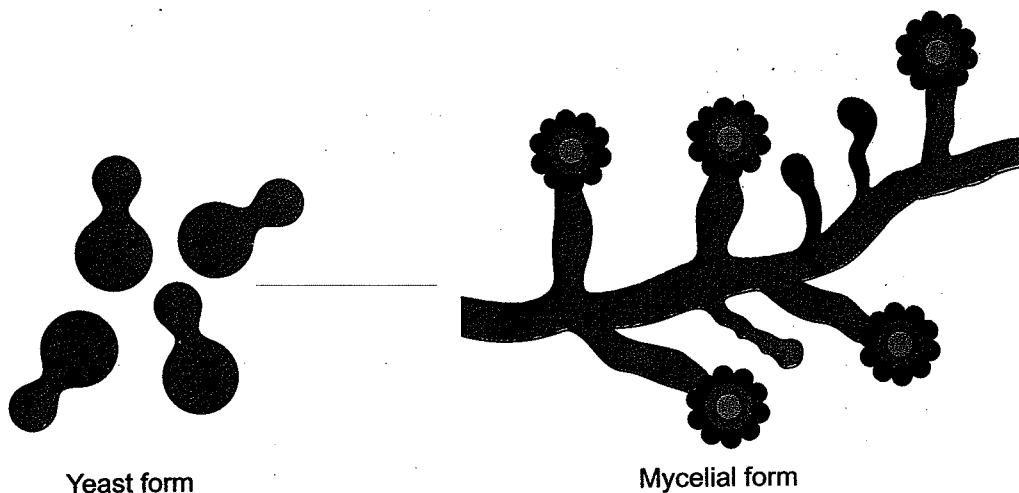


Fig. 73.7 *Histoplasma capsulatum* a) Yeast form, b) Mycelial form.

- **Confirmation of isolate** is done by:
 - Conversion of mycelial form to yeast form by using BHI blood agar at 37°C
 - Exoantigen test—H and M Ag
 - Nucleic acid hybridisation test

Ab Detection

The following tests are used:

- Latex agglutination
- Immunodiffusion
- Complement fixation test
- Four-fold rise in antibody titre is significant
- Immunodiffusion shows two major types of bands, H and M
 - H—suggests active infection
 - M—suggests acute, chronic infection or previous skin test

Skin Test

- It is delayed hypersensitivity test. When antigen is injected intradermally on forearm, after 48 hours, development of area of induration about 5 mm in diameter indicates positive test
- Positive test indicates past or present infection, however negative test does not rule out histoplasmosis

How is histoplasmosis treated?

Histoplasmosis is treated with amphotericin B, ketoconazole, itraconazole, fluconazole and voriconazole.

What causes dubosii histoplasmosis? How is it different from *H. capsulatum*?

It is African histoplasmosis and is caused by variant *duboisii*. It is restricted to central Africa

- It differs from *H. capsulatum* in the following features:
 - Shows large thick-walled yeast
 - Rarely causes pulmonary involvement
 - Extrapulmonary sites such as skin and mucosa are commonly involved

Write a short note on blastomycosis.

- Blastomycosis is a systemic fungal infection that primarily involves lungs, caused by a thermally dimorphic fungus - *Blastomyces dermatitidis*. It is also called **Chicago's disease** and **North American blastomycosis**
- *B. dermatitidis* is a dimorphic fungus and it exists in yeast form at 37°C and in mycelial form at 25°C.
- Blastomycosis is mainly prevalent in North American continent. In India, two cases have been reported from Delhi and Madhya Pradesh
- *B. dermatitidis* is present in soil, infection occurs through inhalation of conidia, rarely through skin.
- Males affected more than females, more common in age group of 20 to 50 years.

Clinical features

- **Pulmonary form**
 - It presents as productive cough, muscle-joint pain or pleuritic chest pain or asymptomatic.

- Chronic disease resembles to carcinoma or pulmonary tuberculosis and disseminate to other organs
- **Cutaneous form**
 - Infection is due to inoculation of fungus by trauma, usually seen in laboratory workers.
 - Lesion is a papule or nodule which breaks down to form fistula discharging purulent material
- **Disseminated form**
 - This form is usually found in immunocompromised hosts due to AIDS and transplant recipients. It is present with chronic pulmonary form and disseminates to organs by haematogenous route forming abscesses. It can involve bones, genitourinary organs
- **Miscellaneous form**
 - Laryngeal Blastomycosis—characterized by hoarseness, pain, dysphagia and shows ulcerative fungating mass as of carcinoma
 - Osteomyelitis—involves vertebra, pelvis, skull, ribs, long bones and cause diffuse or focal osteomyelitis may cause arthritis

Laboratory diagnosis

Specimens

Sputum, pus, biopsy of affected tissue are collected by using standard procedures and transported.

Microscopic Examination (Fig. 73.8)

- **KOH and histopathological study:** They show thick, double-walled multinucleated yeast with single broad-based budding of size 8–10 μm in diameter

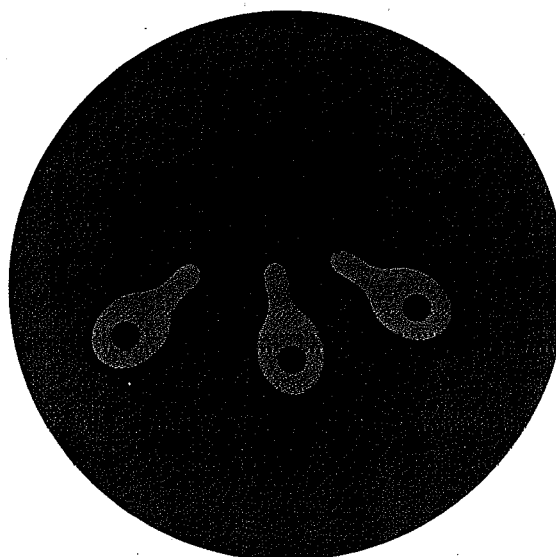


Fig. 73.8 *Blastomyces dermatitidis*.

Culture

- **Media:** Sabouraud's dextrose agar, brain-heart infusion agar, blood agar. Two media inoculated and incubated—one at 25°C and another at 37°C. Cultures show growth after 4–5 weeks
- **Colony on media at 25°C:** Yeast-like initially, becomes fluffy, tan-coloured colony
 - **LCB:** Mycelium with finely branched, septate hyphae show oval, round or pyriform conidia of 2–10 μ in diameter on short terminal and lateral branches. Thick-walled chlamydospores are also seen
- **Colony on media at 37°C:** Cream coloured, smooth, yeast-like colony
 - **LCB:** Thick walled, spherical with single broad-based budding
- **Confirmation of isolate** is done by:
 - Conversion of mycelial form to yeast form by using blood agar at 37°C
 - Exoantigen analysis—A antigen

Skin Test

It is delayed hypersensitivity to blastomycin, not of much value in diagnosis.

Ab Detection

- Complement fixation test
- Precipitation—Immunodiffusion

- ELISA
- RIA

Nucleic Acid Detection by DNA probes.

Animal Pathogenicity

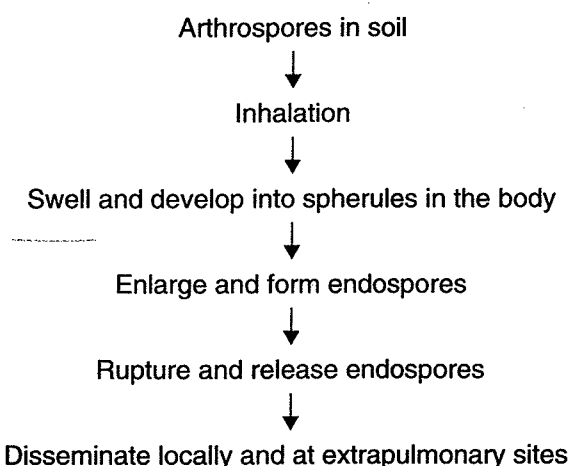
Mice and rats are used to study virulence.

■ Which drugs are recommended for treating blastomycosis?

Amphotericin B and ketoconazole can be used for treating blastomycosis.

■ Write a short note on coccidioidomycosis.

- It is a systemic fungal infection primarily involving respiratory tract caused by a dimorphic fungus *Coccidioides immitis*. Also called valley fever and California disease
- *C. immitis* is a dimorphic fungus.
- In the body it exists as a double walled spherule and in the soil, it grows as a mould
- Soil is the source of infection and infection occurs by inhalation of arthrospores.
- Development of infection by *C. immitis* resulting in coccidioidomycosis is presented in Flowchart 73.1.



Flowchart 73.1. Developmental cycle of coccidioidomycosis.

- **Clinical forms** of coccidioidomycosis are as follows:
- **Pulmonary form** –It is generally asymptomatic. Symptomatic infection presents as a self-limited influenza-like fever, headache, cough, rash and myalgia. It may present as pneumonia, rash and arthralgia
- **Chronic meningitis** –Extrapulmonary form usually manifests as chronic meningitis
- **Disseminated form**
 - The disease may spread beyond lungs
 - It can affect any part except gastrointestinal tract
 - Dissemination occurs through blood
 - It spreads to skin forming granuloma or abscess with local lymphangitis and lymphadenopathy
 - It can spread to bones (ribs, skull and vertebrae) causing osteomyelitis
 - It also involves eyes, larynx, epididymis and central nervous system

Laboratory diagnosis

C. immitis is a highly infectious organism, hence should be handled in biosafety cabinet.

Specimens

- Sputum, respiratory secretions, pus, CSF, biopsy specimens.

Direct Microscopy (Fig. 73.9)

- **KOH mount and calcofluor white stain:** To demonstrate thick-walled, doubly refractile spherules 30–60 μm with and without endospores in specimens
- Histopathological examination

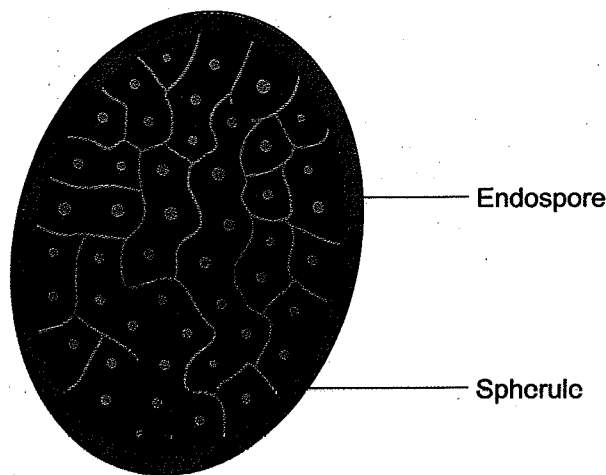


Fig. 73.9 *Coccidioides immitis* – spherule.

H and E, PAS-stained sections show thick-walled, doubly refractile spherules with and without endospores, ruptured spherules releasing endospores.

- Immunofluorescence

Ab conjugated with fluorescent tag is used to identify fungus.

Culture

- **Media:** Sabouraud's dextrose agar, brain–heart infusion agar, blood agar
- Two slants inoculated and incubated—one at 25°C and another at 37°C. Grows in a week
- **Colony on media at 25°C**—whitish cottony colony in 3–10 days
- **Colony on media at 37°C**—no yeast form in routine culture, remains in mould form
- **LCB**—branching, septate hyphae, hyphae with alternate thick-walled barrel-shaped arthrospores and empty cells. Arthrospores are thick-walled and rectangular in shape
- Alternate cells undergo lysis forming chains and releasing arthrospores

Identification

- Mycelial to yeast—conversion by animal inoculation
- Exoantigen test—HS and HL Ag can be detected by gel diffusion technique

Ab Detection

- Complement fixation test
- Precipitation—Immunodiffusion
- IgM—ELISA
- Latex agglutination

Nucleic Acid Detection by DNA probes.

Skin Test

It is a delayed hypersensitivity reaction to coccidioidin, not of much value in diagnosis

Animal Pathogenicity

Mice, rabbit, guinea pig may be used for isolation and culture.

Treatment

Drugs that can be used for treatment are amphotericin B and ketoconazole.

■ Write a short note on paracoccidioidomycosis.

- Paracoccidioidomycosis is a systemic fungal infection primarily involving respiratory tract, caused by *Paracoccidioides brasiliensis*. Formerly it was known as South American Blastomycosis or Brazilian blastomycosis
- *P. brasiliensis* is a dimorphic fungus. Exists as a multibudding yeast at 37°C and mould at 25°C.
- Soil is the source of infection and infection occurs by inhalation of spores. Not transmitted from man to man.
- Males affected more than females, probably because of oestrogen, seen in the age group of 20 to 50 years
- **Clinical forms** of paracoccidioidomycosis are as follows:
- **Pulmonary paracoccidioidomycosis**
 - It is usually asymptomatic. Primary lesions in lung manifest as respiratory disease—spreads to spleen, liver, skin, mucous membrane—may occur by haematogenous route
- **Mucocutaneous paracoccidioidomycosis**
 - It has predilection for cooler areas of body such as nasal and oropharyngeal. Ulcerative granulomatous lesions are seen in mouth, on lips, tongue and conjunctiva
- **Lymphatic paracoccidioidomycosis**
 - It usually manifests as cervical lymphadenopathy and may spread to other regional lymph nodes
- **Disseminated paracoccidioidomycosis**
 - Disease may spread to other organs specially adrenals - common in immunocompromised hosts

Laboratory diagnosis

Specimens

- Sputum, pus, biopsies from lesions, bronchoalveolar lavage.

Direct Microscopy (Fig. 73.10)

- **KOH mount and calcofluor white:**
For demonstration of characteristic yeast cells showing **Mickey Mouse cap** appearance or **mariner's wheel** appearance
- Typical budding yeast of size 15–30 μ with multiple budding at periphery, results in daughter bud attached by narrow base and 2–5 μ in size
- Histopathological Examination

H and E, PAS stained sections show similar findings as above.

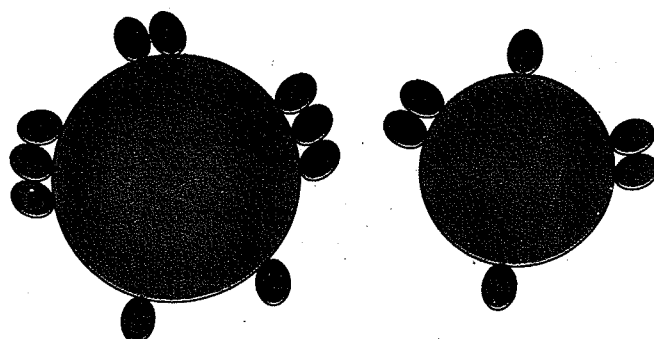


Fig. 73.10 *Paracoccidioides brasiliensis*.

Culture

- **Media:** Sabouraud's dextrose agar, brain-heart infusion agar, blood agar with antibiotics and actidione
- Two slants inoculated and incubated, one at 25°C and another at 37°C. It grows slowly
- **Colony on media at 25°C:** Floccose, leathery, velvety, flat or wrinkled, pink to brown with yellow brown reverse
 - LCB—many times produces sterile hyphae; many intercalary chlamydospores and globose conidia may be seen
- **Colony on media at 37°C:** Cream pasty
 - LCB—yeast cells reproducing by multiple budding, mother cells surrounded by multiple peripheral cells with thin-necked buds
 - Appear like—mariner's wheel or pilot wheel or Mickey Mouse

Identification

- Mycelial to yeast—conversion on brain-heart infusion blood agar
- Exoantigen test—1, 2 and 3 can be detected by gel diffusion technique

Ab Detection

- Complement fixation test
- Precipitation—Immunodiffusion
- Counter immunoelectrophoresis
- ELISA

Ag Detection by ELISA and Immunoblots

Nucleic Acid Detection by DNA probes and PCR

Skin Test

Paracoccidioidin is used as Ag by intradermal injection, not of much value in diagnosis

Animal Pathogenicity

Mice, rabbit and guinea pig may be used for study purposes.

Treatment

The drugs used for treating paracoccidioidomycosis are itraconazole, fluconazole with amphotericin B, and ketoconazole.

74

Chapter

Opportunistic Mycoses

✓ 3N Explain opportunistic mycoses.

- Opportunistic mycoses are the infections found in patients with underlying predisposing conditions, such as weakened immune system, cancer, leukaemia, immunosuppressive therapy, AIDS, etc.
- Opportunistic mycoses include:
 - Candidiasis
 - Cryptococcosis
 - Aspergillosis
 - Zygomycosis
 - Pneumocystosis

✓ 3N Write a note on Aspergillosis.

Aspergillosis is a superficial and systemic fungal infection found in immunocompromised as well as immunocompetent individuals, caused by several species of the genus *Aspergillus*.

- Genus *Aspergillus* consists of many species and three of them are frequently encountered as clinical isolates. These are:
 - *A. niger*
 - *A. flavus*
 - *A. fumigatus*
- *A. fumigatus* is the commonest isolate
- Other species are *A. terreus*, *A. glaucus*, *A. nidulans*, and *A. clavatus*

They are ubiquitous in nature, common as saprobe in soil and decaying plants. Entry - through inhalation and wounds.

✓ Clinical Infections

Clinical infections caused by *Aspergillus* spp. include following:

1. Lung Infections

- These are classified into following three types: Allergic aspergillosis, Aspergilloma and Invasive aspergillosis
- All these infections manifest as variable degree of dyspnoea, cough and expectoration and fever

Allergic aspergillosis

- In atopics, hypersensitivity to fungus manifests as asthma with pulmonary eosinophilia or extrinsic allergic alveolitis and allergic bronchopulmonary aspergillosis (ABPA); both these cause progressive lung damage
- Fungus grows in airways, forms mucous plug containing mycelia, which may block part of lobe or coughed out in ABPA

Aspergilloma

- Fungus colonizes tubercular or other preexisting cavity forming fungal ball
- Fungal ball is a compact mass of mycelia surrounded by fibrous wall
- Usually it is single and of variable size (8–10 cm) and casts radiological shadow

Invasive aspergillosis

- It is usually observed in immunocompromised hosts
- Widespread growth occurs in lungs that may disseminate to various organs, especially kidneys and brain

2. Central Nervous System Infections

It is due to haematogenous dissemination from lung tissues, especially in immunocompromised hosts. It may present as meningitis, meningoencephalitis, abscess and granuloma

3. Paranasal Sinus Infections

Colonization of paranasal sinuses is called paranasal sinus aspergillosis. It may be of different variety as allergic, noninvasive, invasive and fulminant infections

4. Cutaneous Infections

It may be primary or secondary - primary infection is due to direct external inoculation through surgery sites or catheters, which may form papule, ulcer, plaque or nodule. Secondary infection is usually on chest wall in patients of pulmonary aspergillosis due to dissemination. This is more common in immunocompromised hosts

5. Endocarditis

It is seen in immunocompromised hosts and in those who had undergone prior cardiac surgery. Fungal vegetations are formed on heart valves. Patient comes with fever, multiple embolic strokes due to vegetations and their embolization

6. Miscellaneous Infections

It may present as onychomycosis, keratomycosis and otomycosis. Also occurs as mastoiditis and osteomyelitis

✓ Laboratory diagnosis**Specimens**

Sputum, bronchoalveolar lavage, transbronchial biopsy, pus, skin biopsy.

Microscopic Examination

- **KOH preparation:** Direct examination of samples and biopsy by using KOH preparation—shows hyaline septate hyphae with dichotomous branching
- **Calcofluor white (CFW) stain:** shows fluorescence if fungal elements are present
- **Fluorescent antibody technique:** used to detect fungus in smears prepared from samples by Abs conjugated with fluorescent dye
- **Histopathological sections:** Slides stained with H & E, PAS and GMS stain

Culture

- SDA with antibiotics (but without cycloheximide)—two slants are inoculated, one incubated at room temperature and another at 37°C. Culture is examined daily for first week and twice a week after that. Colony characters and LCB picture is described in Table 74.1 (see also Fig. 74.1)

Table 74.1 Colony characters and LCB picture of *Aspergillus* spp.

Species	Colony	LCB
<i>A. flavus</i>	O—velvety, yellow to green or brown R—golden to red brown	Rough, pitted, spiny conidiophores, uniseriate phialides cover entire vesicle, point out in all directions, conidia are yellow-green
<i>A. fumigatus</i>	O—velvety or powdery, smoky green R—white to tan	Smooth conidiophore, uniseriate phialides cover upper half of vesicle parallel to axis of stalk, conidia are blue-green
<i>A. niger</i>	O—wooly, initially white to yellow, becomes brown-black R—white to yellow	Conidiophore of variable length biserial phialides cover entire vesicle forming radiate head, conidia are black

LCB = lactophenol cotton blue, O = obverse, R = reverse.

- Other media used are—Czapek–Dox agar, potato dextrose agar, and malt extract agar. They help in identification of fungus

Ab detection by immunodiffusion and ELISA

Ag Detection

Ags can be detected in serum, urine and other body fluids by:

- Latex agglutination
- ELISA
- RIA
- Immunoblotting

Nucleic Acid Detection by DNA probes and PCR

Skin Test

Used for patients with ABPA, atopic dermatitis and asthma.

Animal Pathogenicity

To study lesions of aspergillosis—Mice and rabbits are used.

Treatment

Drugs that can be used in the treatment of aspergillosis are Amphotericin B and Itraconazole

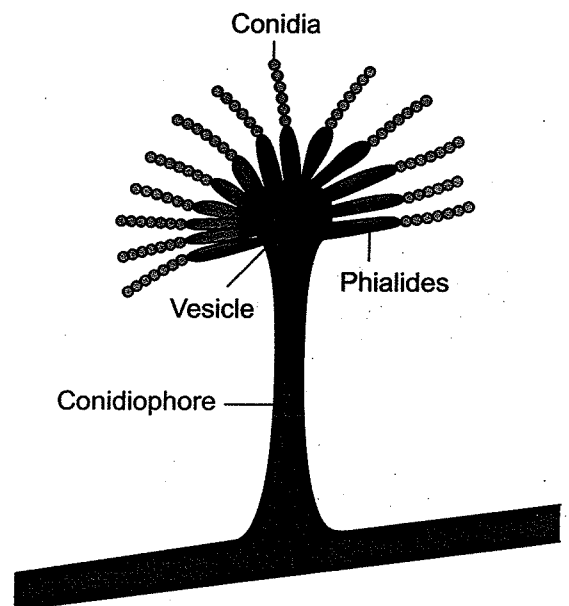
■ Write a note on zygomycosis.

Zygomycosis is an opportunistic fungal infection caused by many aseptate filamentous fungi. It includes mucormycosis and entomophthoromycosis.

- **Mucormycosis** caused by *Rhizopus* spp., *Mucor* spp., *Absidia* spp., *Cunninghamella* and *Apophysomyces*
- **Entomophthoromycosis** caused by *Conidiobolus* and *Basidiobolus*

Morphological Characteristics

- Generally they show
 - Nonseptate hyphae, sporangiophore with sporangium-containing sporangiospores (sexual spores)
 - Also form zygospores (sexual spores)

**Fig. 74.1** *Aspergillus* spp.

- Nature of spore bearing structure, branching of sporangiophores, location of rhizoids, presence of apophysis and shape of columella are used for identification.

Mucor (Fig. 74.2a)

- Branched sporangiophores are present
- Rhizoids absent
- Columella not distinct

Rhizopus (Fig. 74.2b)

- Distinct rhizoids just below sporangiophores
- Ovoid columella

Absidia (Fig. 74.2c)

- Rhizoids are present at nodes not directly below the sporangiophores
- Pear-shaped columella
- Spherical or ovoid sporangiospores

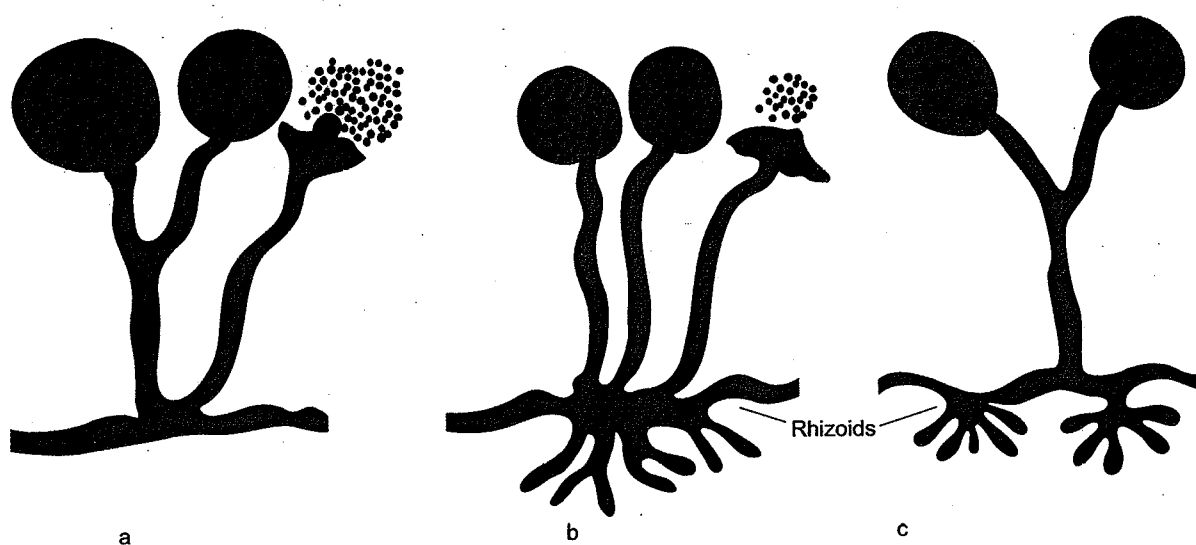


Fig. 74.2 Zygomycetes: (a) *Mucor*, (b) *Rhizopus* and (c) *Absidia*.

Apophysomyces

Similar to *Absidia* but has prominent apophyses.

Cunninghamella

Few globose sporangia at the tip of the sporangiophore.

■ Discuss the pathogenicity of zygomycetes.

The diseases caused by zygomycetes are of following forms:

1. Rhinocerebral

- Most common form - usually found in patients of diabetes, hyperglycaemia, ketoacidosis, leukaemia and lymphoma

- Spreads from nose to brain where it causes invasion of blood vessels. Clinically presents as facial pain, headache, brown and blood stained nasal discharge, black eschar on palate and proptosis and ptosis

2. Pulmonary

- Occurs due to inhalation of spores -found in patients with haematologic malignancy and bone marrow transplantation
- Causes invasion of blood vessel to destruct lung parenchyma causing pulmonary infarction. Clinically presents as chest pain, dyspnoea, haemoptysis

3. Cutaneous

- Primary form - due to direct inoculation through skin
 - Found in patients with severe burn, ulcer in diabetes, and gangrenous cellulitis following trauma
 - Clinically, the patient has painful lesions, ecchymotic areas and leg ulcers
- Secondary form—is due to haematogenous dissemination

4. Gastrointestinal

- Commonly involves stomach, then ileum, caecum, and colon - found in patients with extreme malnutrition and also following surgery of abdominal trauma and contaminated ileostomy. Ulceration of mucosa is feature
- Clinically presents as abdominal pain, diarrhoea, hematemesis, malena

5. Disseminated

- Enters usually through trauma or wound leading to invasion of blood vessels
- By haematogenous dissemination—can involve lung, kidneys, GIT, heart, brain

Laboratory diagnosis

Specimens

- Collected as per the affected site—Nasal discharge, pus, sputum, and biopsy.

Microscopic Examination

- KOH preparation and biopsy with H & E stain show broad, aseptate, ribbon-like hyphae, branching at 45°–90° angles and at irregular intervals
- Presence in tissue is more reliable in proving pathogenicity

Culture

- Two SDA without cycloheximide are inoculated—one is incubated at 25°C and another at 37°C. Repeated isolation is necessary to prove pathogenicity
- Colony characters and LCB picture are help in identification (Table 74.2)

Treatment

Zygomycosis can be treated with amphotericin B.

■ Write a note on pneumocystosis.

Pneumocystosis is an opportunistic fungal infection characterized by involvement of respiratory tract in the form of interstitial plasma cell pneumonia caused by a fungus *Pneumocystis carinii*. It is also called *Pneumocystis carinii* pneumonia (PCP) or atypical pneumonia.

Table 74.2 Colony characters and LCB picture of zygomycetes

Fungus	Colony	LCB picture
<i>Mucor</i>	Gray-brown mycelial colony	Broad aseptate hyphae Branched sporangiophores Spherical, thin-walled sporangium, Rhizoids are absent
<i>Rhizopus</i>	Light to dark gray mycelial colony	Broad aseptate hyphae Sporangiophore single or in groups Sporangium with distinct columella Rhizoids just below conidiophore
<i>Absidia</i>	Light to dark gray mycelial colony	Broad aseptate hyphae Sporangiophore single or in groups Rhizoids not beneath conidiophores Apophyses and columella present

LCB = lactophenol cotton blue.

Aetiology

- *P. carinii*—was observed first by Antonio Carinii as cyst-like structure in lungs, therefore called *P. carinii*
- It has few characters like fungi and few like protozoa, viz.
 - It can be stained with fungal stains. Produces chitin and chitinase, can attack cell wall
 - Has cyst wall as in protozoa. Cannot grow in fungal culture media
 - It is sensitive to antiprotozoal drugs.
 - Absence of ergosterol in cytoplasmic membrane and nucleic acid study shows its similarity to fungi; it is therefore classified as fungi

Route of Entry

Droplet inhalation.

Predisposing Factors

- AIDS
- Persons with impaired CMI
- Malignancy
- Cytotoxic or steroid therapy
- Protein energy malnutrition
- Premature infants and old age

■ Describe the morphological forms of *Pneumocystis carinii*.

Pneumocystis carinii has following three morphological forms:

1. Trophozoite
2. Cyst
3. Sporozoite

Stage between trophozoite and cyst is called **precyst (sporocyst)**.

Trophozoite

Present in lung alveoli.

Pleomorphic, 2–5 μ in size and are in clusters.

Covered with tubular projections which help in attachment.
Possesses thin fragile, flexible external layer.

Cyst

Present in clinical specimens.

Large, 4–6 μ , oval, thick-walled containing up to 8 sporozoites.

After release of sporozoites from cyst, empty cysts stain black with GMS.

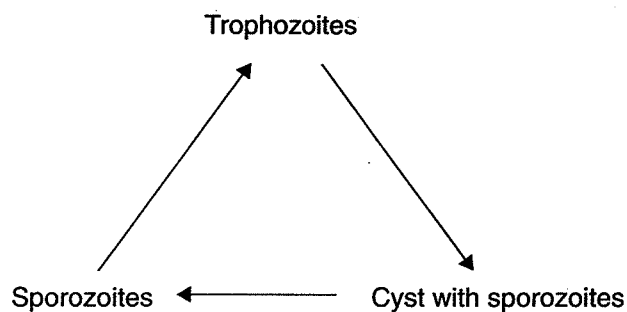
Sporozoites

Oval, amoeboid, 1–2 μ .

When stained with Giemsa, show red purple nuclei and basophilic cytoplasm.

After release, they get converted into trophozoites.

Lifecycle of *P. carinii* can be diagrammatically presented as shown below:



Clinical Features

- *P. carinii* causes pneumocystosis; also called primary atypical pneumonia and *P. carinii* pneumonia (PCP)
 - Respiratory pneumocystosis: Disease presents as increasing nonproductive cough, dyspnoea, fever, tachypnoea, occasional haemoptysis, chest pain, chills, night sweats.
 - Nonrespiration pneumocytosis: It may involve lymph nodes, bone marrow, spleen and liver in AIDS patients

Laboratory diagnosis

Specimens

Bronchoalveolar lavage, induced sputum, tissue biopsy, expectorated sputum - collected as per standard guidelines and transported as early as possible.

Microscopic Examination

- GMS shows masses of black coloured organisms.
- Phase contrast microscope with stain can be used
- Giemsa shows organisms with reddish nucleus and blue cytoplasm
- Immunofluorescence by using Abs to cyst and trophozoites
- Calcofluor white stain can also be used

Culture

Not useful.

Nucleic Acid Detection by PCR and DNA probes

Ab Detection

Nonspecific—as Abs are seen in healthy individuals also.

- CFT
- ELISA
- Immunofluorescence test

Treatment

Pneumocystosis can be treated with Trimethoprim + Sulfamethoxazole.

75

Chapter

Miscellaneous Mycoses

- **Define keratomycosis. Mention its aetiology, predisposing factors, and clinical features.**

Definition

An invasive fungal infection of cornea is called keratomycosis or mycotic keratitis.

Aetiology

The causal agents are:

- *Aspergillus fumigatus*
- *A. flavus*
- *Fusarium* spp.
- *Acremonium* spp.
- *Penicillium* spp.
- *Candida* spp.
- *Pseudallescheria boydii*
- *Curvularia* spp.
- *Bipolaris* spp.
- *Alternaria* spp.

Predisposing Factors

- Corneal trauma with vegetative material contaminated with fungi
- Injudicious use of topical steroids and antibiotics
- Surgery like keratoplasty
- Contact lens wearers

Clinical Features

- Foreign body sensation in eye
- Photophobia
- Blurred vision
- Corneal ulcer

- **Describe the laboratory diagnosis of keratomycosis.**

Specimen

Corneal scrapings.

Collection

Corneal scrapings are collected with no. 15 Bard Parker blade from base and margins of ulcer aseptically using local anaesthetic.

Microscopic Examination

- **KOH preparation:** Wet preparation shows presence of fungal elements
- **Gram stain:** It shows Gram-positive hyphae, pseudohyphae and yeast cells
- **Calcofluor white:** This can also be helpful, specially when sample contains scanty fungal elements and examined with fluorescent microscope

Culture

- Two sets of SDA are inoculated and incubated at 25°C and 37°C, respectively
- Cultures are observed for 2–3 weeks; colony characters and LCB mount help in identification of fungus

■ How can keratomycosis be treated?

Keratomycosis can be treated by using following agents:

- Natamycin ointment
- Flucytosine drops
- Miconazole drops

■ Define otomycosis. Mention its aetiology and clinical features.**Definition**

Otomycosis is a superficial fungal infection of external auditory canal.

Aetiology

- *Aspergillus fumigatus*
- *A. niger*
- *A. terreus*
- *Penicillium* spp.
- *Pseudoallescheria boydii*
- *Candida* spp.

Clinical Features

Itching, irritation, discomfort, pain and discharge.

■ How can otomycosis be diagnosed in a laboratory?**Specimen**

Ear swab from debris.

Collection

Ear swab is collected with cotton swab.

Microscopic Examination

- **KOH preparation:** Wet preparation shows presence of fungal elements
- **Gram stain:** It shows Gram-positive hyphae, pseudohyphae and yeast cells

Culture

- Two sets of SDA are inoculated and incubated at 25°C and 37°C respectively
- Cultures are observed for 2–3 weeks; colony characters and LCB mount help in identification of fungus

■ How would you treat otomycosis?

Otomycosis can be treated with topical application of Hamycin, Nystatin and Imidazole.

■ Write in brief about mycotoxicosis.

- Diseases resulting due to ingestion of food contaminated with mycotoxin (fungal toxins) are called mycotoxicosis
- Mycotoxins, their corresponding fungi and source are presented in Table 75.1

Table 75.1 Mycotoxins causing agents

Mycotoxins	Fungus	Source
1. Aflatoxin	<i>Aspergillus flavus</i>	Nuts, Maize
2. Fumonisin	<i>Fusarium</i> spp.	Maize
3. Ochratoxins	<i>Aspergillus</i> spp.	Cereals
	<i>Penicillium</i> spp.	Bread
4. Trichothecenes	<i>Fusarium</i> spp.	Maize
5. Cyclopiazonic acid	<i>Aspergillus flavus</i>	Groundnut, Corn
	<i>Penicillium cyclopium</i>	

Clinical Features

Mycotoxins are the cause of mycotoxicosis. Aflatoxin is highly toxic to animals and birds, and probably to human beings also. It can cause hepatomas in ducklings and rats. It may have carcinogenic effects for humans. Mycotoxins contain alkaloids which cause marked peripheral vasoconstriction that leads to necrosis and gangrene.

■ Define mycetismus. What are its clinical features?

Definition

Clinical disease produced by ingestion of toxins along with fungus is known as mycetismus. It is also called mycetism, muscarinism and mushroom poisoning.

Clinical Features

It may range from minor gastrointestinal disturbances to hallucinations and delirium.

Examples

1. *Claviceps* spp. causes ergot poisoning
2. *Coprine* spp. causes coprine poisoning
3. *Inocybe* spp. causes muscarine poisoning
4. *Amanita* spp. causes cyclopeptides poisoning

UNIT

VI

Parasitology

76

Chapter

Introduction to Parasitology

■ What is Parasitology? What does Medical Parasitology deal with?

- **Parasitology** is the branch of microbiology that deals with the study of parasites
- **Medical parasitology** is the branch of parasitology that deals with the study of parasites, which infect human beings and the disease they produce

■ Define the terms: Parasite and Host.

- **Parasite:** These are the organisms that infect other living beings. They live in or on the body of another living being and obtain shelter and nourishment from it
- **Host:** The organism, which harbours parasite, is known as host

■ Mention the different classes of parasites.

The different classes of parasites are as follows:

1. **Ectoparasites**—parasites, which live outside on the surface of the body without penetrating into the tissues, e.g. lice, ticks, mites, etc.
2. **Endoparasites**—parasites, which live inside the body of the host in blood, tissues, body cavities and other organs, e.g. all protozoan and helminthic parasites of humans

■ Mention the different types of host.

The different types of host are as follows:

1. **Definitive host**—the host in which the adult stage of parasite lives or the sexual mode of reproduction takes place is called the definitive host
2. **Intermediate host**—the host in which the larval stage of parasite lives or the asexual mode of reproduction takes place is called the intermediate host
3. **Reservoir host**—a host, an invertebrate species, in which the parasite passes its lifecycle and which may act as the source of infection for humans is called a reservoir host

■ Comment on the nomenclature of parasites.

- Each parasite possesses two names:
 - A **generic name**—which begins with an initial capital letter, e.g. *Ascaris*
 - A **specific name**—which begins with an initial small letter, e.g. *Ascaris lumbricoides*
 - The name should be *italicized*

■ Describe in brief the morphological features of protozoan parasites.

- They are unicellular organisms with one or more nuclei. A single cell contains all structures required to perform various functions
- The multiplication occurs by binary fission or multiple fission or by sexual means
- Protozoan parasites consists of cytoplasm and nucleus.

- **Cytoplasm:** Differentiated into
 - **Ectoplasm**—External hyaline part, homogenous in nature, which functions as
 - organ of locomotion, participates in engulfment of food material, respiration, discharging waste material and
 - acts as protective covering
 - **Endoplasm**—Internal granular portion that participates in nutrition and reproduction

Structures developed from ectoplasm

1. Locomotory organs

- (a) **Pseudopodia:** These are temporary projections of the cytoplasm used as organ of locomotion and also participates in engulfment of food material, e.g. Rhizopodea.
- (b) **Flagella:** These are long, delicate thread-like filaments arising from cytoplasm, one to eight in number and act as organ of locomotion, e.g. Zoomastigophora.
- (c) **Cilia:** These are fine hair-like filaments covering the entire body act as organ of locomotion, e.g. Ciliata.

Structures located in endoplasm

1. **Nucleus:** Situated inside the endoplasm. Usually single, but may be two or more than two in number, e.g. in ciliata, two nuclei—a small, micronucleus and a large, macronucleus. Surrounded by tough nuclear membrane. Shows one or more nucleoli or a central endosome or kinetoplast. Chromatin may be distributed along the inner surface of the nuclear membrane or as condensed masses around the karyosome. Its structure helps in differentiation of the genera and species

Functions

Controls various activities of parasite and regulates the reproduction

2. **Contractile vacuoles:** Situated inside the endoplasm - serve to regulate the osmotic pressure
3. **Kinetoplast:** Certain protozoan parasites, e.g. haemoflagellates show the presence of non-nuclear DNA containing round or rod shaped, or tiny dot-like body known as kinetoplast in addition to the nucleus.

■ Comment on trophozoite and cyst stages of protozoan parasites.

1. Trophozoite

- Derived from Greek word *trophos* meaning nourishment
- It is an actively feeding and growing stage of the protozoan parasite
- It can be converted into an inactive stage known as cyst

2. Cyst Stage

- It is an inactive stage of the protozoan parasite
- It is resistant to abnormal environmental conditions, hence able to survive under the unfavourable conditions
- It is infective to its human host and reaches to new host by mechanical transfer, i.e. with the help of carriers or by some agency, e.g. by house flies to food and drink

Classify protozoan parasites.

Based on the organ of locomotion (motility), pathogenic protozoa are classified into the following four types:

1. **Rhizopoda:** Protozoa, which are motile with the help of pseudopodia, e.g. *Entamoeba histolytica*—causative agent of amoebiasis—amoebic dysentery, liver abscess and abscess in other organs

2. **Mastigophora:** Protozoa, which are motile with the help of flagella. These are of the following two types:
 - **Intestinal flagellates**—Flagellates infecting intestine and vagina, e.g. *Giardia lamblia* and *Trichomonas vaginalis*.
 - **Haemoflagellates**—Flagellates infecting blood and tissues, e.g. *Leishmania* spp. and *Trypanosoma* spp.
3. **Sporozoa:** Protozoa, which are nonmotile, e.g. malarial parasites (*Plasmodium* spp.), *Toxoplasma* spp.
4. **Ciliata:** Protozoa, which are motile by cilia, e.g. *Balantidium coli*

■ What are helminths? What are different groups of helminths?

- The helminths (helminths – worms) are worm-like, metazoan parasites, that are multicellular and bilaterally symmetrical and have three germ layers (triploblastic metazoan).
- Medically important helminths are categorized into two main groups:
 - **Platyhelminthes**—(platy – flat) include cestodes and trematodes
 - **Nemathelminthes**—(nema – thread) include nematodes

■ Write the differences between cestodes, trematodes and nematodes.

The important differentiating features are summarized in Table 76.1.

■ Write the general features of cestodes.

- Cestodes are tape-like segmented worms with no separate sexes
- Size: Few millimeters to several meters
- Occur in three different morphological forms, viz.
 - **Adult worm**—found in the intestine of definitive host-man and animal
 - **Eggs**—laid by adult worms passed in faeces
 - **Larval stage**—seen in intermediate host
- Adult worms have the following three parts:
 - **Head (scolex)**—provided with slit-like grooves or cup-like suckers and in some species hooks are present on scolex, which serve as organs of attachment
 - **Neck**—is the region behind the scolex
 - **Strobila (body or trunk)**—consists of a series of segments called proglottides

Table 76.1 Important differences between cestodes, trematodes and nematodes

Features	Cestodes	Trematodes	Nematodes
Shape	Tape like	Leaf like	Elongated, cylindrical
Segmentation of body	Segmented	Unsegmented	Unsegmented
Sexes	Not separate—hermaphrodite (monoecious)	Not separate—hermaphrodite (monoecious) except schistosomes	Separate (dieocious)
Head end	Suckers present rostellum with hooks in some species	Suckers present, no hooks	No suckers, no hooks Head end is pointed than caudal end
Alimentary canal	Absent	Incomplete, no anus	Complete, anus present Some species have well developed buccal capsule
Body cavity	Absent	Absent	Present

Proglottides are formed continuously during the entire course of life of the adult worm. The newly formed proglottides are small and immature. The process of maturation progresses as the immature segments are pushed away from the neck due to formation of new proglottides. Mature proglottides contain fully-developed reproductive organs (well-developed reproductive system). Each mature segment contains ovaries and testes (monoecious). Eggs are produced by self-fertilization or cross fertilization. Eggs containing proglottides are the **gravid segments**

- No body cavity and no mouth or digestive system is present. Food is absorbed through its body surface
- They have simple excretory system

■ Mention the important cestodes infecting humans.

Cestodes infecting humans are classified into the following two types:

1. **Pseudophyllidean cestodes**—cestodes possessing false or slit-like grooves
 - *Diphyllobothrium latum* (fish tapeworm) causing diphylobothriasis
2. **Cyclophyllidean cestodes**—cestodes possessing cup-like round suckers
 - *Taenia solium* (pork tapeworm) causing taeniasis or cysticercosis
 - *T. saginata* (beef tapeworm) causing taeniasis
 - *Echinococcus granulosus* (dog tapeworm) causing unilocular hydatid disease
 - *Hymenolepis nana* (dwarf tapeworm) causing hymenolepiasis

■ Mention the general features of trematodes.

- Trematodes are leaf-like—unsegmented, flat worms, digenetic (sexual and asexual generations) and are called **flukes**
- Size varies from 1 mm to several centimetres in length
- Sexes are not separate (monoecious) except the schistosomes, which are diecious
- Head is with cup-shaped suckers but no hooks are present. Suckers serve as organs of attachment
- Body cavity is absent
- The alimentary canal present but incomplete, anus is absent
- Excretory and nervous systems are present. Well developed and complete reproductive system is present
- They occur in three different morphological forms:
 - **Adult worm**—in definitive host, generally man
 - **Eggs**—laid by adult worms gain access into the water
 - **Larval stage**—develops in water and enters to proper intermediate host (snail or crabs) for further development
- The worm is oviparous—liberates eggs. Eggs are operculated (except in schistosomes) and develop only in water
- Eggs do not float in saturated salt solution

■ Classify important trematodes that infect humans.

Important trematodes infecting humans are as follows:

1. **Blood trematodes** (blood flukes): *Schistosoma haematobium*, *Schistosoma mansoni*, *Schistosoma japonicum*
2. **Hepatic trematodes** (liver flukes): *Fasciola hepatica*, *F. gigantica*, *Clonorchis sinensis*, *Opisthorchis* spp.
3. **Intestinal trematodes** (intestinal flukes): *Fasciolopsis buski*, *Heterophyes heterophyes*, *Metagonimus yokogawai*, *Watsonius watsoni*, *Gastrodiscoides hominis*
4. **Lung trematodes** (lung flukes): *Paragonimus westermani*

■ Mention the general features of nematodes.

- Nematodes are elongated, cylindrical, unsegmented worms with separate sexes (diecious). Male is smaller than female and their posterior end is curved ventrally
- Size varies from 5 mm to one meter
- They possess a tough, acellular, hyaline cuticle
- They have a body cavity in which the various organs float
- Digestive system is complete and consists of mouth, oesophagus, intestine and anus
- They are diecious, i.e. sexes are separate
- Females are either viviparous (produce larvae), oviparous (lay eggs) or ovoviviparous (lay eggs, which hatch immediately producing larvae)
- Moulting (shedding of the old cuticle) is a feature in the development of nematode larvae

■ Classify nematodes.

Nematodes are classified into two types based on the habitat of the adult worms. These are as follows:

1. Intestinal nematodes
2. Tissue nematodes
 - Intestinal nematodes
 - Nematodes inhabiting the intestine of definitive host
 - Man is the optimum host for all the nematodes
 - Only one host is required for intestinal nematodes
 - Occur in three morphological forms: **Adult worm**—found in intestine of man, **Eggs**—passed in stool, **Larvae**—develop from egg
 - Tissue nematodes
 - Nematodes invading tissues and organs
 - Require two hosts to complete their lifecycle
 - Intermediate host is required for larval development
 - Occur in two morphological forms: **Adult worm**—in definitive host, **Larval stage**—in insect vector or cyclopes

■ Enumerate intestinal nematodes found in human beings.

Following are the intestinal nematodes found in humans:

- *Ascaris lumbricoides*
- *Ancylostoma duodenale* and *Necator americanus*
- *Strongyloides stercoralis*
- *Trichuris trichiura*
- *Enterobius vermicularis*
- *Trichinella spiralis*

■ Enumerate the medically important tissue nematodes.

Following are the medically important tissue nematodes:

1. *Wuchereria bancrofti*
2. *Brugia malayi*
3. *Loa loa*
4. *Onchocerca volvulus*
5. *Dracunculus medinensis*

77

Chapter

Medically Important Amoebae

■ Enumerate the protozoa causing intestinal infections.

Entamoeba histolytica is an important cause of intestinal amoebiasis. The other protozoa causing intestinal infections are:

- *Giardia lamblia*
- *Balantidium coli*
- *Isospora belli*
- *Cryptosporidium parvum*

■ Discuss in brief the morphology, lifecycle, pathogenicity and laboratory diagnosis of *Entamoeba histolytica*.

Morphology

It occurs in the following three stages:

1. **Trophozoite or vegetative form (feeding stage of parasite; Fig. 77.1a)**
 - Shape: Irregular
 - Size: 10–40 μ (average 20–30 μ)
 - Motility: Actively motile with the help of pseudopodium
 - Cytoplasm: Differentiated into: *Ectoplasm*—thin, clear, translucent outer layer and *Endoplasm*—granular inner layer containing nucleus, food vacuoles red blood cells, occasionally white blood cells and tissue debris
 - Nucleus: Spherical, 4–6 μ in size, contains small dot-like structure, central in position called **karyosome**, which is surrounded by clear halo. Nucleus is surrounded by a nuclear membrane lined with a single layer of uniformly distributed chromatin granules.
2. **Precystic stage (Fig. 77.1b)**
 - It is a transitory stage, formed during the conversion of trophozoite to cyst
 - Shape: Round or oval with blunt pseudopodium
 - Size: 10–20 μ
 - Endoplasm: Free of red blood cells and other ingested particles
 - Nucleus: Retains the features of trophozoite stage
3. **Cyst stage (Fig. 77.1c)**
 - Shape: Spherical
 - Size: 10–15 μ
 - Cytoplasm: A mass of glycogen and 1–4 cigar-shaped or oblong refractile rods known as chromatoid bodies are present in immature cysts. As it matures, the glycogen mass and chromatoid bodies disappear
 - Nucleus: Early cyst contains single nucleus. The nucleus undergoes two successive mitotic divisions to form 2 and finally 4 nuclei in mature cysts (quadrinucleate)

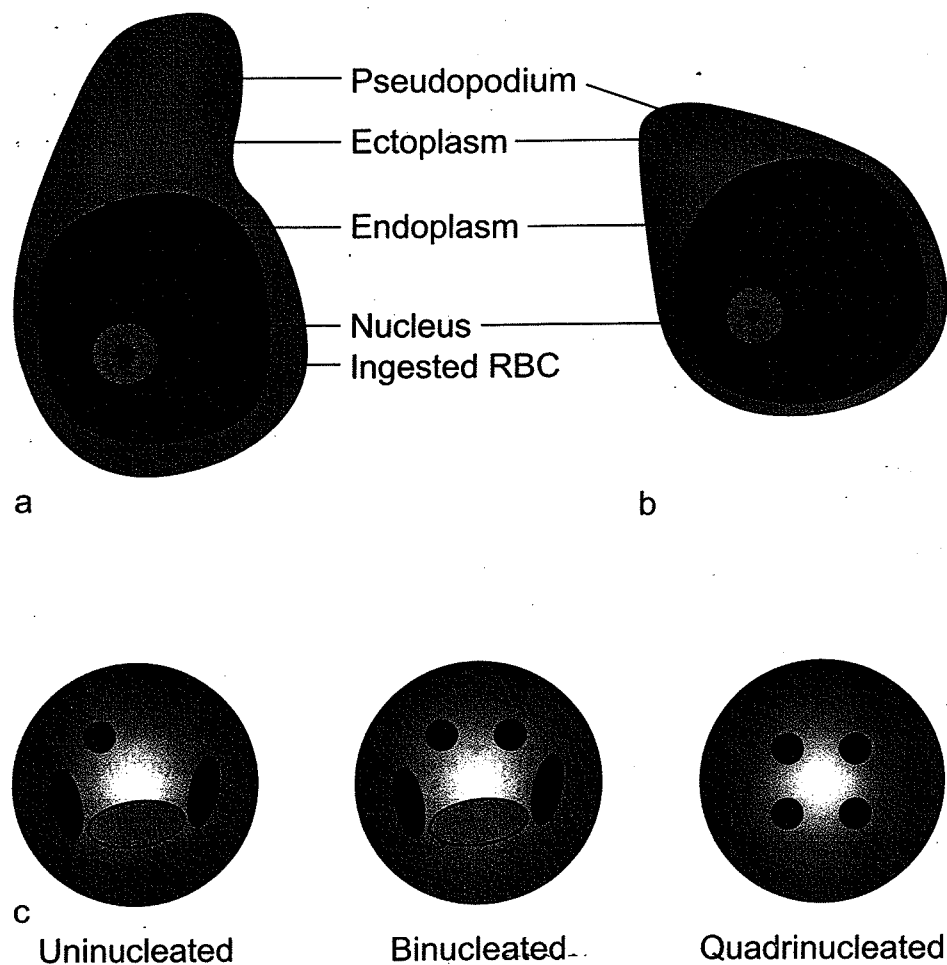


Fig. 77.1 *Entamoeba histolytica*: (a) trophozoite stage, (b) precystic stage and (c) cyst stages.

Lifecycle (Flowchart 77.1)

Definitive host: Human beings

Intermediate host: Not required, passes lifecycle in one host—the man

Pathogenicity

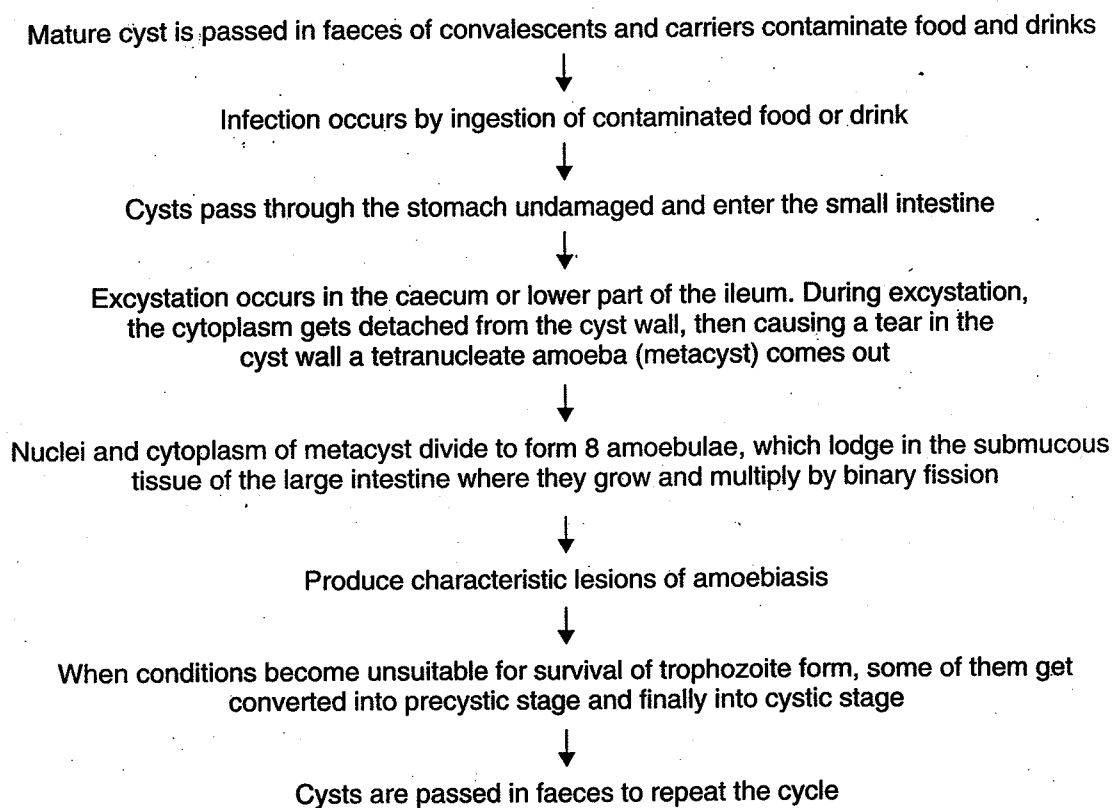
Trophozoites in lumen do not cause any illness, when they invade the intestinal tissue disease is caused (Flowchart 77.2). This happens in 10% cases, 90% cases remain asymptomatic.

Ulcers

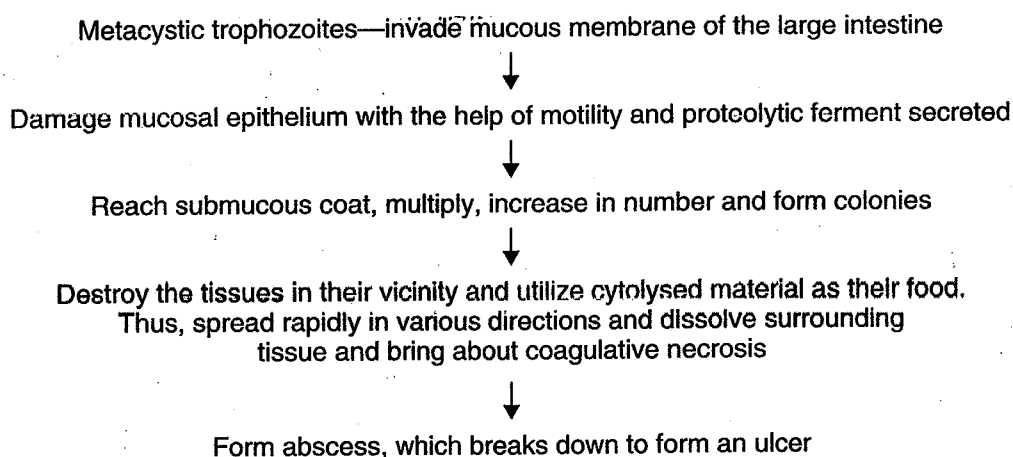
- Multiple and confined to the colon
- Size: Pinhead to one inch or more
- Shape: Round or oval, flask shaped in cross-section
- Margin: Ragged or undermined
- Base: Generally formed by the muscular coat and filled up by the necrotic material, yellowish or blackish slough

Spread to other organs

- During the invasion of intestinal wall, amoebae penetrate the radicals of portal vein and transported to the liver
- Some of them get established in the hepatic lobules where they multiply and initiate lytic necrosis with inflammatory reaction



Flowchart 77.1 Lifecycle of *Entamoeba histolytica*.



Flowchart 77.2 Disease development in infections by *Entamoeba histolytica*.

- Cause obstruction to the circulation and produce thrombosis of portal venules that result in anaemic necrosis of the surrounding liver cells (miliary abscesses)
- Miliary abscesses coalesce to form an abscess (few millimeters to few centimeters) containing thick chocolate brown pus (anchovy sauce pus-containing liquefied necrotic liver tissue and blood)
- Untreated abscess may rupture into the surrounding organs and tissues such as lung, pleural cavity, pericardium, peritoneal cavity, stomach, intestine, inferior vena cava, abdominal wall and skin
- Spread to lung and skin may also occur by haematogenous route (via blood) and amoebae may also spread to organs such as brain, kidneys, spleen, and adrenals by haematogenous route

Clinical Features

The disease caused is known as **amoebiasis**. It is of two types:

- Intestinal amoebiasis—also known as primary amoebiasis
- Extraintestinal amoebiasis—also known as secondary or metastatic amoebiasis

Intestinal amoebiasis is characterized by:

- The passage blood and mucus in stool
- The stool is foul smelling and brownish black in colour
- The patient is usually afebrile and nontoxic
- Sometimes only diarrhoea and vague abdominal symptoms

Extraintestinal amoebiasis

- *Hepatic amoebiasis*—occurs in tropics in 2–10% cases of intestinal amoebiasis. It is characterized by:
 - The pain and tenderness in the right hypochondrium
 - Fever with chills
 - Shoulder pain due to irritation of phrenic nerve
 - Weight loss
 - Jaundice is infrequent
- Also causes pulmonary amoebiasis, cutaneous amoebiasis, cerebral amoebiasis, splenic abscess, amoebic vaginitis and amoebic ulcer of penis rarely

Laboratory Diagnosis

1. Diagnosis of intestinal amoebiasis (amoebic dysentery)

Amoebic dysentery has to be differentiated from bacillary dysentery by inspecting stool sample macroscopically and microscopically. The differentiating features are given in Table 77.1.

Table 77.1 Differentiating features of amoebic and bacillary dysentery

Features	Amoebic dysentery	Bacillary dysentery
Macroscopic features		
Number of motions	6–8 per day	More than 10 per day
Odour of stool	Offensive	Odourless
Colour of stool	Dark red	Bright red colour
Nature of stool	Mixed with blood and mucous	Blood and mucous, no faeces
Consistency	Not adherent to container	Adherent to container
Reaction	Acidic	Alkaline
Microscopic features		
Red blood cells	Reddish-yellow colour, in clumps	Bright red colour, discrete or in rouleaux
Pus cells	Scanty	Numerous
Macrophages	A few	Several, few with ingested RBCs
Eosinophils	Present	Absent
Charcot–Leyden crystals	Present	Absent
Motile bacteria	Present	Absent
Amoebae in stool	Present	Absent

The following steps are used for the diagnosis of intestinal amoebiasis (amoebic dysentery):

- **Collection and transport of specimen:** Faeces are collected and transported immediately.
- **Microscopy**
 - **Saline preparation**—to demonstrate motile trophozoite
 - **Iodine preparation**—to demonstrate trophozoite in killed state and cysts in stained state
 - **Detection of amoebic antigen**—in stool sample by using CIEP and by ELISA using monoclonal antibody
 - **Serological tests**—negative for antibodies in early cases and in the absence of deep invasion

2. Diagnosis of hepatic amoebiasis

- Pus aspirated from liver or liver biopsy is observed for trophozoites
- Stool sample may show cysts in about 15% cases
- **Serological tests:** Positive test indicates the presence of specific Abs in the blood. The Abs can be detected by CFT, agar gel diffusion, latex agglutination, indirect haemagglutination test, CIEP, ELISA, immobilization test and indirect immunofluorescence test
- **Detection of antigens:** The Ag can be detected by RIA. Amoebic antigens in serum are detected by using the ELISA, CIEP and coagglutination tests

3. Diagnosis of pulmonary amoebiasis

Demonstration of trophozoites in anchovy sauce sputum. The *E. gingivalis* normally present in mouth may cause confusion in diagnosis. Diagnosis by serological tests.

4. Newer techniques in the diagnosis of amoebiasis: Molecular methods

- Detection of *E. histolytica* DNA in stool sample by using DNA hybridization probe
- Polymerase chain reaction (PCR) to detect amoebic genome in pus, stool, etc.

Treatment

- Metronidazole—drug of choice - effective in intestinal as well as extraintestinal amoebiasis
- Diloxanide fuorate, diiodohydroxyquin and paromomycin—active against trophozoites as well as cysts - effective in cyst passer, as they kill cysts and prevent recurrence
- In amoebic colitis and amoebic liver abscess—metronidazole, tinidazole, secnidazole and ornidazole are effective
- Chloroquine, tinidazole, emetine hydrochloride, tetracycline, etc. are effective against trophozoites in tissue in hepatic and pulmonary amoebiasis

■ Write a short note on pathogenic free-living amoebae.

- Free-living amoebae are aerobic amoebae present in soil, water and mud
- Free-living amoebae are called amphizoic amoebae because of their ability to multiply in the body of host (endozoic) as well as in free living conditions (exzoic)
- Some species are pathogenic to humans, e.g. *Naegleria* spp. and *Acanthamoeba* spp.

Naegleria spp.

It has two species, viz., *N. fowleri* and *N. gruberi*. Only *N. fowleri* is pathogenic. It occurs as trophozoite and cyst.

Trophozoite is of the following two forms:

1. Amoeboid form (Fig. 77.2a)

- Size: 10–20 μ
- Shape: Slug-shaped with one broad and other pointed end

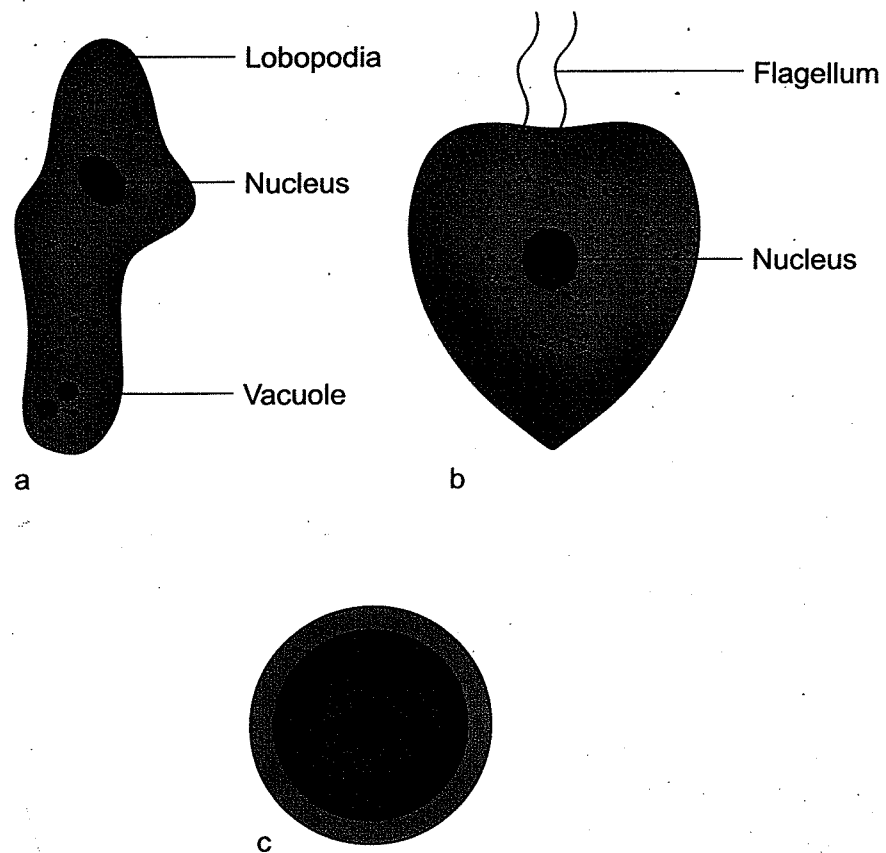


Fig. 77.2 *Naegleria fowleri*: (a) amoeboid, (b) flagellate and (c) cyst forms.

- Motility: Motile with rounded pseudopodia (lobopodia)
- Nucleus: Spherical with a large endosome (karyosome)
- This is replicating form
- 2. **Flagellate form** (Fig. 77.2b)
 - In water, amoeboid form is transferred to nonreplicating flagellate form
 - It is a pear-shaped cell with flagella
 - This form is believed to be more infectious
 - Flagellate form is converted into amoeboid form

Cyst form (Fig. 77.2c)

- Develops from amoeboid form
- Spherical in shape with double cyst wall
- Resistant to drying and other unfavourable conditions
- It is the resting stage and is not formed in host tissue

Pathogenicity

- It causes **primary amoebic meningoencephalitis**
- Infection occurs by nasopharyngeal contamination during swimming or diving in contaminated water, and also by inhalation of decaying animal manure, which supports growth of this parasite
- Characterized by meningism, cranial nerve palsies and severe headache. Disease ends fatally within a week
- It also causes allergic pneumonitis and humidifier fever

Laboratory Diagnosis

- By demonstrating the trophozoites in cerebrospinal fluid or in brain tissue at autopsy
- By culture on proteose peptone glucose medium or non-nutrient agar seeded with *Escherichia coli*
- By intranasal inoculation into mice that leads to meningoencephalitis and death within 5 days

Treatment

Amphotericin B and sulphadiazine.

Acanthamoeba (*Hartmannella*)

It occurs in the following two forms:

- **Trophozoite:** Large, 20–50 μ , with spine-like pseudopodia (acanthopodia) and large nucleus with distinct karyosome (Fig. 77.3a). No flagellate stage, as in *Naegleria* spp.
- **Cyst:** Formed in tissues. It is polygonal, thick-walled and highly resistant (Fig. 77.3b)

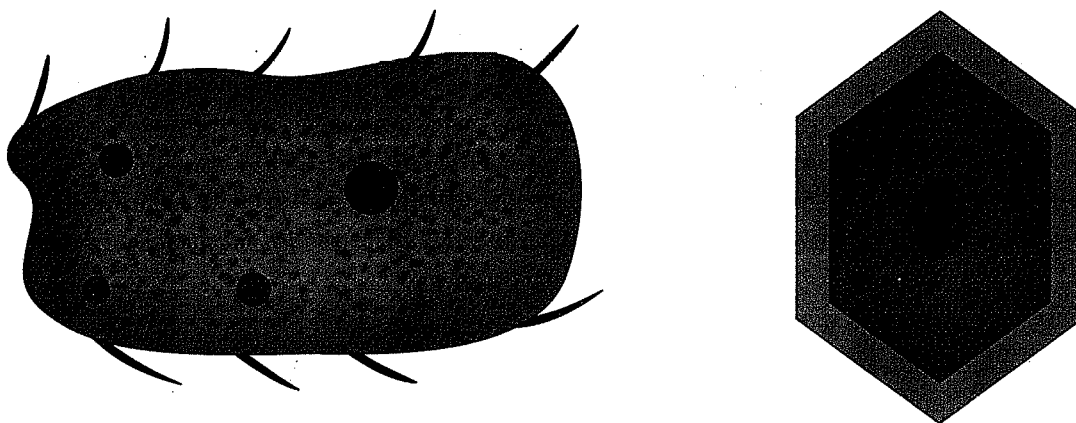


Fig. 77.3 *Acanthamoeba*: (a) trophozoite and (b) cyst forms.

Pathogenicity

- It causes **granulomatous amoebic meningoencephalitis**
- Predisposing factors—chronically ill patients, debilitated patients, patients with SLE and AIDS and patients on steroid or chemotherapy
- *A. culbertsoni*—common species. *A. polyphaga*, *A. castellani* and *A. astromyxis* rarely involved
- Infection occurs by ingestion, inhalation or through traumatized skin or eyes
- It also causes acanthamoebiasis—granulomatous lesions in the skin, lungs, eyes, middle ear, gastric mucosa, etc.
- Also causes chronic amoebic keratitis, especially in contact lens users and allergic pneumonitis

Laboratory Diagnosis

- By demonstration of trophozoites in cerebrospinal fluid
- By histopathology—in biopsy specimens
- By culture on agar seeded with *E. coli*
- For diagnosis of keratitis—demonstration of cysts in corneal scrapings by wet mount, histology or culture

Treatment

Propamidine, chlorhexidine, miconazole, ketoconazole for keratitis or keratouveitis. No effective treatment for meningoencephalitis

■ Write the features that differentiate *Entamoeba histolytica* from *Entamoeba coli*.

Entamoeba coli is a nonpathogenic amoeba, normally present in the intestinal tract. Its medical significance is that it has to be differentiated from *Entamoeba histolytica*. The differences between *Entamoeba histolytica* and *Entamoeba coli* are summarized in Table 77.2 (see also Fig. 77.4a and b).

Table 77.2 Differentiating features between *Entamoeba histolytica* and *Entamoeba coli*

<i>Entamoeba histolytica</i>	<i>Entamoeba coli</i>
Trophozoite	
1. Size: 20–40 μ	20–50 μ
2. Motility: Actively motile	Sluggishly motile
3. Haematophagous (ingest RBCs)	Nonhaematophagous
4. No ingested bacteria	Ingest bacteria
5. Cytoplasm: Clearly defined into endoplasm and ectoplasm	Not defined, ectoplasm rarely seen
Cyst	
1. Size: 6–15 μ	15–20 μ
2. Karyosome: Central in position	Eccentric in position
3. Nucleus number: 1–4	1–8

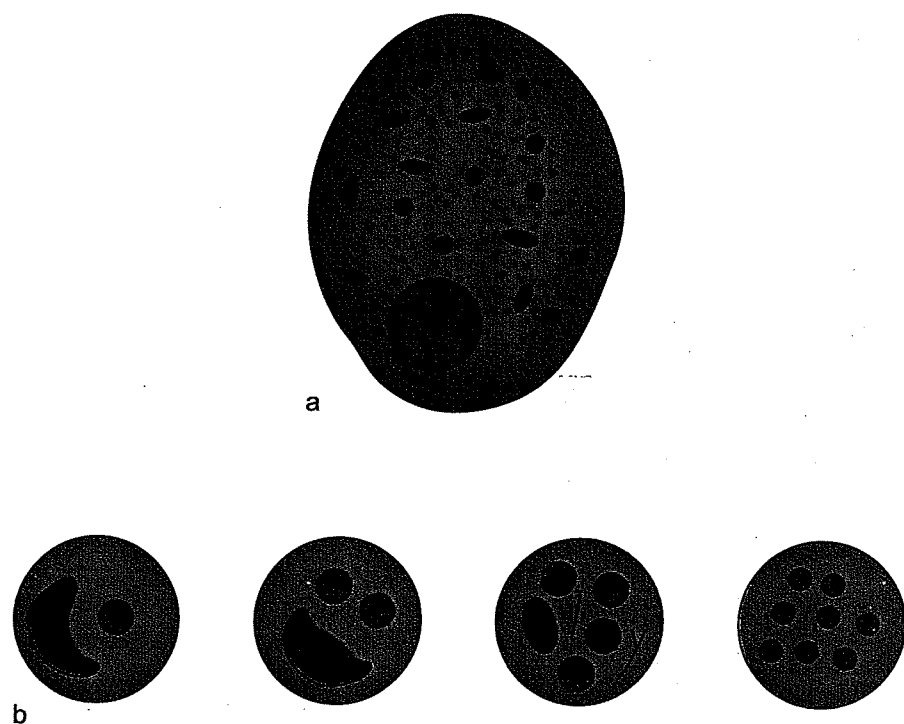


Fig. 77.4 *Entamoeba coli*: (a) trophozoite and (b) cyst forms.

78

Chapter

Medically Important Flagellates

Protozoan parasites motile with the help of flagella are known as flagellates.

■ Classify flagellates on the basis of their habitat.

Based on their habitat in human host, flagellates are classified into three types:

1. **Intestinal flagellates:** flagellates found in the alimentary tracts. Most of them—nonpathogenic commensals and only one, i.e. *Giardia lamblia* is pathogenic.
2. **Oral and vaginal flagellates:** flagellates found in the oral cavity and urogenital tracts. Most of them are nonpathogenic commensals and only one, i.e. *Trichomonas vaginalis* is pathogenic.
3. **Haemoflagellates:** flagellates found in the blood and tissues of human beings and other animals. These include two genera: (a) *Leishmania* spp. and (b) *Trypanosoma* spp.

LSN.

■ Discuss in brief the morphology, lifecycle, pathogenicity and laboratory diagnosis of *Giardia lamblia*.

Morphology

It occurs in the following two forms:

1. **Trophozoite or vegetative form** (Fig. 78.1a)
 - Shape: Tennis or badminton racket or pear shaped
 - Size: $14 \times 7 \mu$
 - Motility: Actively motile with the help of 4 pairs of flagellae arising from axonemes
 - Nucleus: Two nuclei, one on each side. Nucleus has large central endosome with no chromatin granules on the nuclear membrane
 - Axostyles: Slender rod-like structures, two in number, formed by the fusion of axonemes of flagella
 - Symmetry: Bilaterally symmetrical
 - Anterior end: Broad, posterior end—pointed
 - Dorsal surface: convex, ventral surface—concave
 - Sucking disc: Present ventrally on concave surface
2. **Cyst stage** (Fig. 78.1b)
 - Shape: Oval, football shaped
 - Size: $12 \times 7 \mu$
 - Nucleus: 4 nuclei in cluster or lie in pairs at opposite pole
 - Cyst wall: Tough hyaline
 - Axostyles: Two in number, obliquely placed, divides cyst in two equal halves
 - Remains of flagella and sucking disc are present

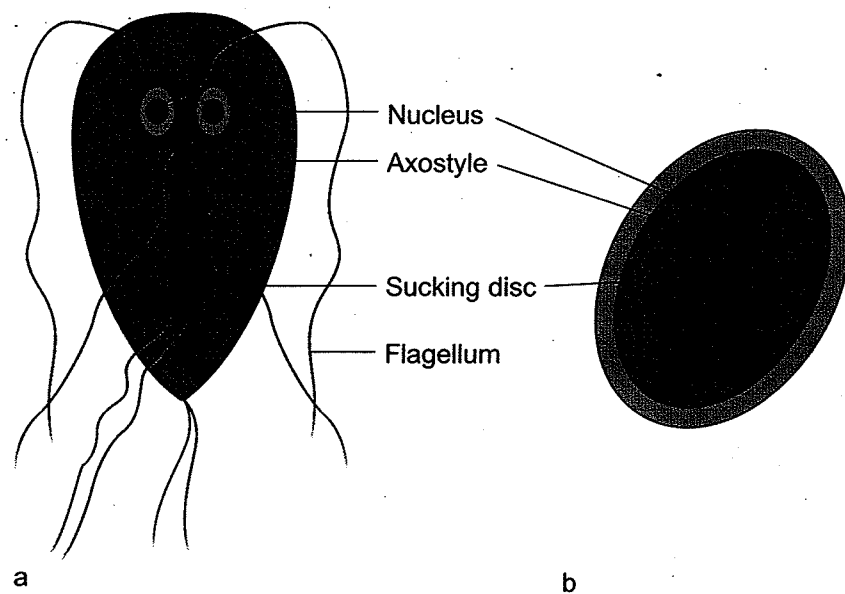


Fig. 78.1 *Giardia lamblia*: (a) trophozoite and (b) cyst.

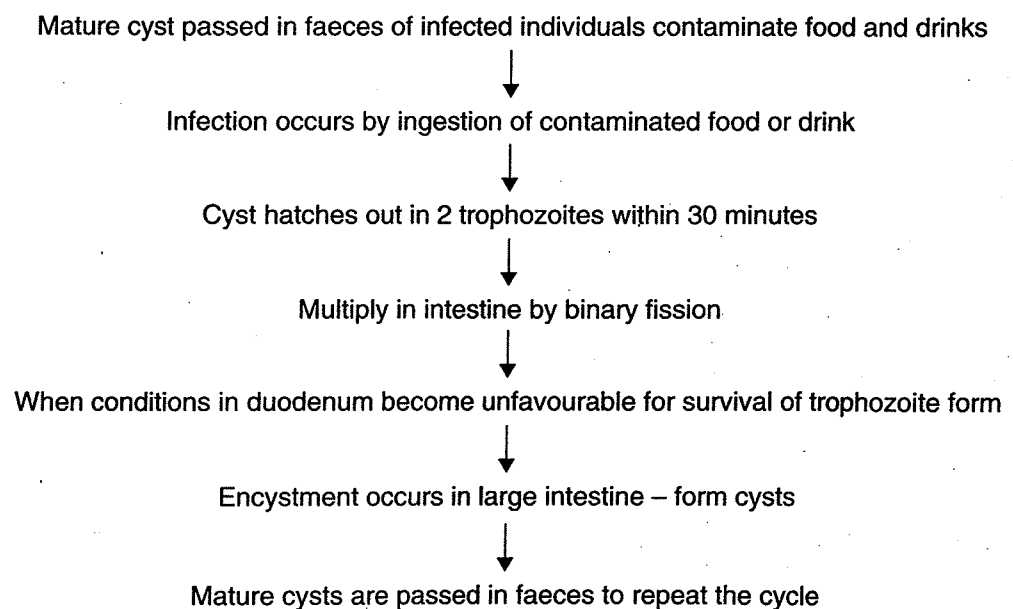
Lifecycle (Flowchart 78.1)

Definitive host: Human beings

Intermediate host: Not required, passes lifecycle in one host

Pathogenicity

It is not a tissue invader. Trophozoites—colonize in the duodenum and attach to epithelial cells with the help of sucking disc. Because of their close association with the epithelium of the upper



Flowchart 78.1 Lifecycle of *Giardia lamblia*.

part of the small intestine, they may cause a mechanical interference in the absorption of fats, fat soluble vitamins and haematinic factors.

Clinical Features

The disease caused is known as **giardiasis**. Clinically, it is manifested as:

- Diarrhoea—5–6 stools a day—explosive watery stools
- Indefinite abdominal pain, nausea, vomiting
- Steatorrhoea—passage of yellowish and greasy stool with excess fat
- Malabsorption
- Severe flatulence
- Cholecystitis and jaundice occasionally
- Fever, anaemia and allergic manifestations
- Acute enterocolitis and chronic enteritis
- Children may develop chronic diarrhoea, malabsorption, weight loss, anaemia, impairment of growth and chronic indigestion

Laboratory Diagnosis

Specimen

Faeces should be collected in clean, wide-mouthed container and transported immediately within 30 minutes to laboratory for examination.

Microscopy

- **Saline preparation**—shows motile trophozoites “falling leaf motility”
- **Iodine preparation**—shows trophozoites in killed state and cysts in stained state. Only cysts - in asymptomatic carriers
- Demonstration of cysts in concentrated stool sample, when cysts are less in number
- Demonstration of trophozoites in bile aspirated from duodenum (bile A) or removed from bile duct (bile B). Bile A can also be obtained by the **string test** or **Enterotest** in which a gelatin capsule containing a nylon string is used. The free end is attached to cheek and capsule is swallowed. A nylon string is exposed in the duodenum after the capsule dissolves. It is allowed to remain there for 4–6 hours. After that, it is removed and the bile-stained mucus is collected on slide and observed for motile trophozoites
- **Giardia specific antigen detection**—by ELISA and direct immunofluorescence test
- **Serological tests**—such as ELISA and indirect immunofluorescence test are used for demonstration of antibodies.

Treatment

Metronidazole and tinidazole—drugs of choice. Atabrine and furazolidone are also effective

■ Discuss in brief the morphology, lifecycle, pathogenicity and laboratory diagnosis of *Trichomonas vaginalis*.

Morphology

It occurs as trophozoite only.

Trophozoite or vegetative form (Fig. 78.2)

- Shape: Pear shaped or ovoid
- Size: 10–30 μ long and 5–10 μ broad
- Nucleus: Single nucleus
- Motility: Wobbling or rotatory movement

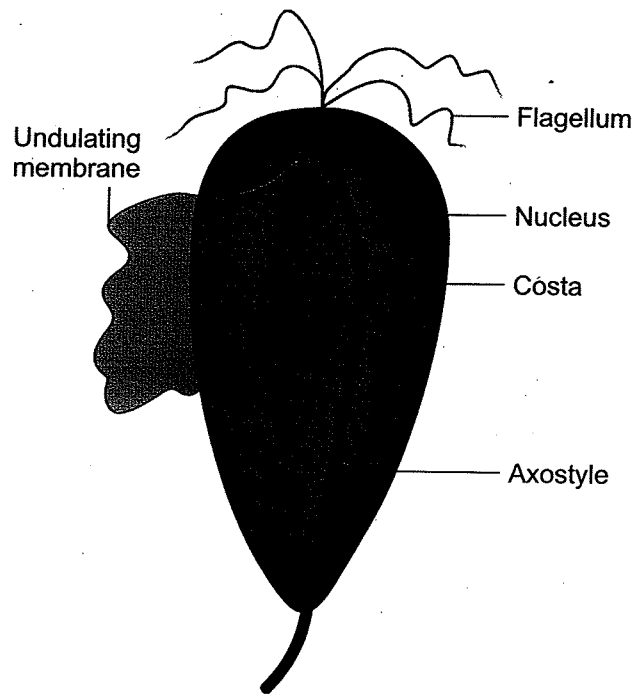


Fig. 78.2 *Trichomonas vaginalis*—Trophozoite.

- Flagella total number 5, 4 directed anteriorly, 1 directed posteriorly forming the margin of an undulating membrane, which is supported at its base by a flexible rod—the costa
- Axostyle: It originates anteriorly and terminates posteriorly as a tail-like appendage
- Cytoplasm shows granules, which are most numerous alongside the axostyle and costa
- Division by binary fission

Lifecycle

Definitive host: Human beings - it inhabits vagina, and cervix in females and urethra, seminal vesicles, epididymis and prostate in males

Intermediate host: Not required, passes lifecycle in one host—the humans

Lifecycle is completed in one host only. The trophozoite passes from person to person by sexual contact. Transmission in female also occurs by the exchange of contaminated towels, underclothing or other toilet articles.

Pathogenicity

The disease caused is known as **trichomoniasis**

- *In females* it causes **vaginitis** characterized by vaginal inflammation, burning and itching sensation and frothy, offensive, yellow vaginal discharge. The vulva and surrounding area may become red and inflamed. Dysuria, urinary frequency and dyspareunia may also occur.
- *In males* the infection is usually asymptomatic. Some patients may develop urethritis or prostatitis

Laboratory Diagnosis

Specimens

Vaginal discharge in females, urethral discharge; prostatic fluid and centrifuged urine sample in males.

- **Direct Microscopy** - Demonstration of motile trophozoites by saline wet mount and in smears stained by Acridine orange stain, Papanicolaou stain, Direct fluorescent antibody stain

- **Antigen detection:** By ELISA test using monoclonal Abs
- **Ab detection:** By indirect haemagglutination test
- **Nucleic acid detection:** By using DNA probes and PCR

■ **Write in short the treatment for *T. vaginalis* infection.**

Metronidazole—drug of choice in both sexes. Simultaneous treatment of sexual partner.

■ **What are haemoflagellates? Enumerate the haemoflagellates found in human.**

- Haemoflagellates are flagellates found in blood and tissues of human beings and other animals
- The haemoflagellates found in human are as follows:
 - *Leishmania donovani*—causing kala azar
 - *L. tropica*—causing oriental sore
 - *L. braziliensis* and *L. mexicana*—causing Espundia
 - *Trypanosoma brucei*—causing African trypanosomiasis (sleeping sickness)
 - *T. cruzi*—causing South American trypanosomiasis (Chagas' disease)

LSN ■ **Discuss in brief the morphology, lifecycle, pathogenicity and laboratory diagnosis of *Leishmania donovani*.**

Morphology

It occurs in the following two stages:

1. **Amastigote form (LD body, leishmanial form or aflagellar stage; Fig. 78.3a)**
 - This stage occurs in vertebrate host (human, dog, hamster). It is intracellular, resides inside the macrophages, monocytes, neutrophils or endothelial cells
 - Shape: Round or oval
 - Size: 2–4 μ along the longitudinal axis
 - Cytoplasm: Pale blue when stained by Romanowsky's stain

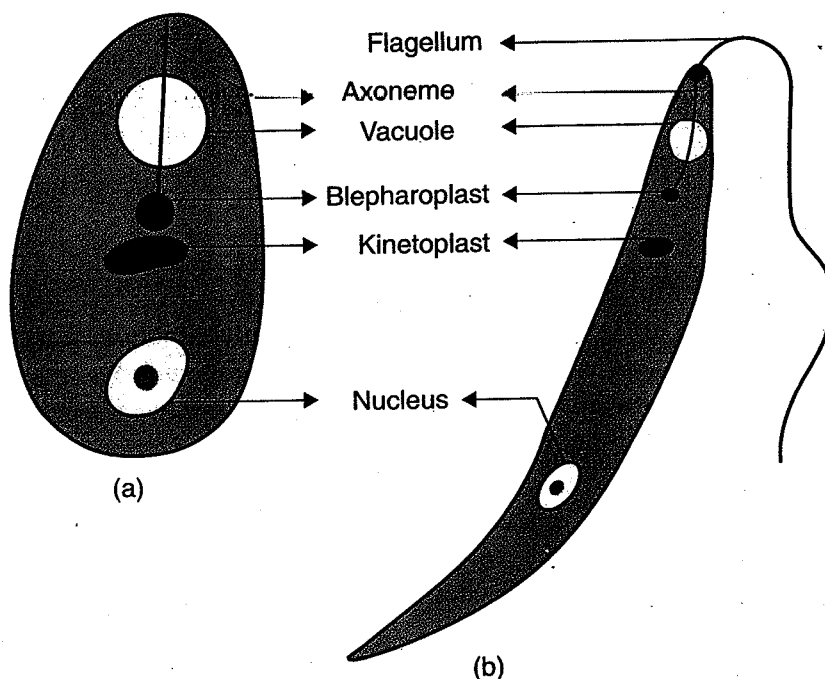


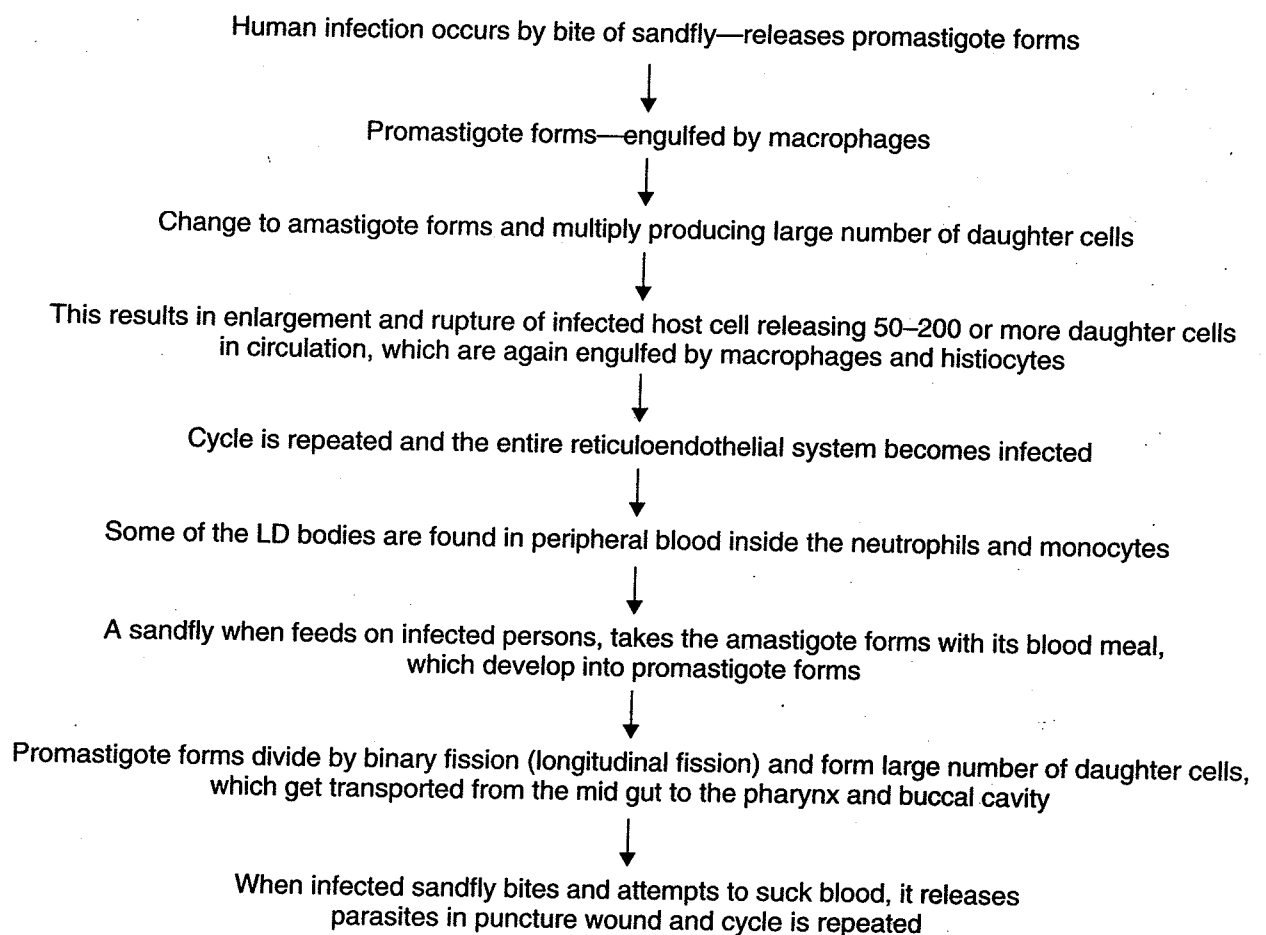
Fig. 78.3 *Leishmania donovani*: (a) amastigote form and (b) promastigote form.

- Nucleus: Large, oval or round, stains red in colour
 - Kinetoplast: Rod-like or tiny dot-like body at right angle to the nucleus, red in colour. It consists a rod-like parabasal body and a dot-like blepharoplast
 - Axoneme: A root of flagellum arising from blepharoplast to margin of body
 - Vacuole: Clear, unstained space lying alongside the axoneme
2. **Promastigote form (leptomonad or flagellar stage; Fig. 78.3b)**
- Shape: Initially short, oval or pear shaped, later on becomes long slender spindle shaped
 - Size: 15–20 μ long and 1–2 μ broad
 - Nucleus: Red, central in position
 - Kinetoplast: It lies transversely near the anterior end
 - Vacuole: Present near the root of flagellum
 - Flagellum: Single flagellum, 15–30 μ in length

Lifecycle (Flowchart 78.2)

Definitive host: Human beings, also dogs in some areas

Intermediate host: Sandfly—*Phlebotomus* spp. (*Phlebotomus argentipes* in India and Pakistan)



Flowchart 78.2 Lifecycle of *Leishmania donovani*.

Pathogenicity

The disease caused is known as kala azar or visceral leishmaniasis. The incubation period is generally 3–6 months, may be very short sometimes—10 days or very long—2 years.

Clinical Features

- Fever—continuous, remittent or irregular type
- Splenomegaly—starts early and progressively enlarges with the progress of disease
- Hepatomegaly and lymphadenopathy may also be seen
- Emaciation and anaemia in fully developed cases
- Skin—becomes dry, rough and darkly pigmented, hence the name kala azar
- Jaundice because of liver damage
- Epistaxis and bleeding gums are common
- In 75–95% of untreated cases, death occurs in about 2 years. Death occurs due to complications such as amoebic or bacillary dysentery, pneumonia, pulmonary tuberculosis, cancrum oris, and other septic infections due to immunosuppressive effect
- **Post kala azar dermal leishmaniasis**—about 10–20% cases recovered from kala azar, develop a nonulcerative cutaneous lesions, which develop after the completion of treatment. These lesions are of three types:
 - Depigmented macules commonly on the trunk and extremities
 - Erythematous patches—on the nose, cheeks and chin having butterfly distribution and are photosensitive becoming prominent during the middle of the day
 - Granulomatous nodules—painless, yellowish pink nodules on skin and mucous membrane of tongue and eyes. Nodules mostly appear on face but may appear on other parts also.

Laboratory Diagnosis

Specimens

Blood, spleen aspirate, bone marrow or biopsy from splenic pulp tissue.

Direct methods

Microscopy: Demonstration of amastigote forms by Romanowsky's stain

Antigen detection: Latex agglutination test to detect Ag in urine of patient.

Nucleic acid detection: By using DNA probes and PCR.

Culture: In Novy–MacNeal–Nicolle (NNN) medium; specimen is inoculated into water of condensation and incubated at 22°C for 1–4 weeks. At the end of every week, a drop of condensation fluid is examined for promastigote forms

Animal inoculation: The specimen is inoculated intraperitoneally or intradermally into hamster and spleen impression smears are observed for presence of parasites as death of animal takes several months

Indirect Methods

Serological tests

1. **Specific tests:** Different serological tests are used to demonstrate specific antibodies using leishmanial antigens prepared from cultures. These tests include:
 - Complement fixation test
 - Indirect haemagglutination test
 - ELISA
 - Counter current immunoelectrophoresis
 - Indirect immunofluorescence test
 - Direct agglutination test
 - Immunochromatographic test
2. **Nonspecific tests**
 - **Complement fixation test with WKK antigen:** It is a nonspecific test that uses antigen prepared from human tubercle bacillus (Ag prepared by Witebsky, Klingenstein and Kuhn). Presently Ag is prepared from Kedrowsky's acid-fast bacillus. This test helps in

early diagnosis (positive in 3 weeks) but it is also positive in tuberculosis, leprosy and pulmonary eosinophilia

- **Other tests:** Infection with *L. donovani* results in hypergammaglobulinaemia, i.e. increase in the level of IgG. These Abs are detected by Napier's aldehyde (formal gel) test and Chopra's antimony test
- **Laboratory diagnosis of dermal leishmaniasis:** It includes the following:
 - Demonstration of amastigote forms in biopsy material obtained from nodular skin lesions by Leishman's stain
 - Skin test (Montenegro): Intradermal injection of 0.1 ml of promastigote antigen, the test is read after 72 hours. It is positive in dermal leishmaniasis and in individuals recovered from kala azar, however, negative in active cases of kala azar

Treatment

Pentavalent antimony compounds such as sodium antimony gluconate, urea stibamine, etc. Synthetic nonmetallic compound such as pentamidine is an alternative. Amphotericin B for drug resistant organisms. In refractory cases, splenectomy followed by chemotherapy may succeed.

■ **Write a short note on *Leishmania tropica*.**

Morphology

It occurs in two stages:

1. **Amastigote form:** This stage resides in the large mononuclear cells of the skin (clasmatocytes) – it is morphologically similar to *L. donovani*
2. **Promastigote form:** Found in sandfly. It is morphologically similar to *L. donovani*

Lifecycle

Lifecycle is similar to *L. donovani*.

Pathogenicity

The disease caused is known as cutaneous leishmaniasis—known as oriental sore, tropical sore or Delhi boil. The incubation period is few weeks to 6 months, may be 2 years.

Clinical Features

Cutaneous leishmaniasis - characterized by papular, crusted, granulomatous eruptions of the skin. The sores are 2 or 3, sometimes single in number and heal spontaneously but slowly—take 6 months. The sores are distributed on the exposed parts of the body—on the face and extremities.

Laboratory Diagnosis

Demonstration of amastigote forms in material obtained from the edge of sore. Skin test (Leishmanin test)—positive in oriental sore

Treatment

Pentavalent antimony compounds and dehydroemetine.

■ **Write short note on *Leishmania braziliensis*.**

Morphology

It occurs in two stages:

1. **Amastigote form** -This stage resides inside the macrophages of skin and mucous membrane of nose and buccal cavity. It is morphologically similar to *L. donovani*
2. **Promastigote form** -Found in sandfly. It is morphologically similar to *L. donovani*

Lifecycle

Lifecycle is similar to *L. donovani*.

Pathogenicity

The disease caused is known as **espundia—mucocutaneous leishmaniasis**.

Clinical Features

Characterized by specific ulcerative granuloma of the skin followed by involvement of the mucocutaneous area in some cases.

Laboratory Diagnosis

Demonstration of amastigote forms in skin and mucocutaneous lesions. Intradermal skin test—using culture of *L. braziliensis*

Treatment

Pentavalent antimony compounds and pyrimethamine or amphotericin B

Ta

M
fo
SP
SN
it
ME
C
Ir
P
ir
T
F

A

79

Chapter

Malarial Parasites

✓ SN: Enumerate the species of Plasmodia causing malaria. Discuss in brief the morphology, lifecycle, pathogenicity and laboratory diagnosis of malarial parasites.

The species of Plasmodia causing malaria are *P. vivax*, *P. falciparum*, *P. malariae* and *P. ovale*

Morphology

The morphological features of four species of *Plasmodium* are summarized in Table 79.1 (Fig. 79.1).

Table 79.1 Distinguishing morphological features of *Plasmodium* species

Morphological form	<i>P. vivax</i>	<i>P. falciparum</i>	<i>P. malariae</i>	<i>P. ovale</i>
Sporozoites	Narrow and slightly curved. Two types: 1. Tachy-fast-develop in PES 2. Brady-slow (hypnozoites)	<ul style="list-style-type: none"> Sickle shaped No hypnozoites 	<ul style="list-style-type: none"> Relatively thick No hypnozoites 	Two types: 1. Develop in PES 2. Hypnozoites
Pre-erythrocytic schizont (PES)				
Size	42 μ in diameter	60 \times 30 μ	Not observed	70–80 μ \times 40–50 μ
No. of merozoites per schizont	10,000–12,000	30,000–50,000	15000 (approximately)	15000
Merozoites	Invade reticulocytes and younger RBCs	Reticulocytes, young and old RBCs	Mature and old RBCs	Young RBCs
Erythrocytic schizogony				
Cycle	48 hours	48 hours	72 hours	48 hours
Invasion of RBC	Each RBC by one parasite	More than one parasite	By one parasite	By one parasite
Percentage of infected RBCs	1–2%	High parasitaemia up to 50%	Less than 1%	1–2%
Trophozoite				
Ring stage	<ul style="list-style-type: none"> Size–2.5–3 μ, large Motile, Cytoplasm–blue, Nucleus–red, Vacuole–unstained 	<ul style="list-style-type: none"> 1.25–1.5 μ, small Ring attached to margin of RBC Form appliqué or accolé form Binucleate rings–common 	<ul style="list-style-type: none"> 2.5 μ Same as <i>P. vivax</i> Thicker and more intensely stained 	<ul style="list-style-type: none"> 2–2.5 μ Same as <i>P. vivax</i>
Amoeboid form	<ul style="list-style-type: none"> Large, irregular 	<ul style="list-style-type: none"> Large, irregular 	<ul style="list-style-type: none"> Band like 	<ul style="list-style-type: none"> Bilobed

Continued

Table 79.1 Distinguishing morphological features of *Plasmodium* species—cont'd

Morphological form	<i>P. vivax</i>	<i>P. falciparum</i>	<i>P. malariae</i>	<i>P. ovale</i>				
	<ul style="list-style-type: none">Accumulates pigmentRBC becomes irregularLoses red colour—becomes colourless	<ul style="list-style-type: none">6–12 brick red Maurer's dotsNo increase in sizeColour—reddish violet	<ul style="list-style-type: none">Dark brown or black pigmentNo increase in sizeNot decolourized	<ul style="list-style-type: none">Oval shapedNormal				
Schizont								
Early schizont	<ul style="list-style-type: none">Size—9–10 μ <p>Round, vacuole disappears, large nucleus lies at the periphery</p>	<ul style="list-style-type: none">4.5–5 μ <p>Not seen in peripheral blood, develop in capillaries of heart, spleen, bone marrow, brain, intestine</p>	<ul style="list-style-type: none">6.5–7 μ <p>Same as in <i>P. vivax</i></p>	<ul style="list-style-type: none">6.2 μ <p>Same as in <i>P. vivax</i></p>				
Late and mature schizont	Nucleus divides to form daughter nuclei and develop into merozoites with central nucleus and surrounding cytoplasm	Same as in <i>P. vivax</i>	Same as in <i>P. vivax</i>	Same as in <i>P. vivax</i>				
Size of merozoites	1.5–1.75 \times 0.75 μ	0.5–0.7 μ in diameter	2–2.5 μ in diameter	2–2.5 μ in diameter				
Number	12–24	18–24	6–12	6–12				
Arrangement	Rosette form	Grape-like cluster	Daisy head appearance	Irregular				
Gametocyte	Male	Female	Male	Female	Male	Female	Male	Female
Size	9–10 μ	10–12 μ	8–10 μ \times 2–3 μ	10–12 μ \times 2–3 μ	7–7.5 μ	7–7.5 μ	9 μ	9 μ
Shape	Spherical	Spherical	Kidney shaped	Crescent shaped	Round	Round	Oval	Oval
Cytoplasm	Light blue	Deep blue	Light blue	Deep blue	Pale blue	Deep blue	Pale blue	Deep blue
Nucleus	Large diffuse	Small compact	Scattered fine granules	Small compact	Large diffuse	Small compact	As in <i>P. vivax</i> (for both male and female)	

Lifecycle of Malarial Parasites

Definitive host: Female anopheline mosquito—sexual phase

Intermediate host: Human—asexual phase

Human Cycle (Flowchart 79.1)

Human infection occurs by bite of infected mosquito, which releases **sporozoites**. The human cycle shows the following stages:

1. Pre-erythrocytic schizogony

- Sporozoites released in blood capillaries reach the liver and enter the parenchymal cells (hepatocytes) to initiate Pre-erythrocytic schizogony

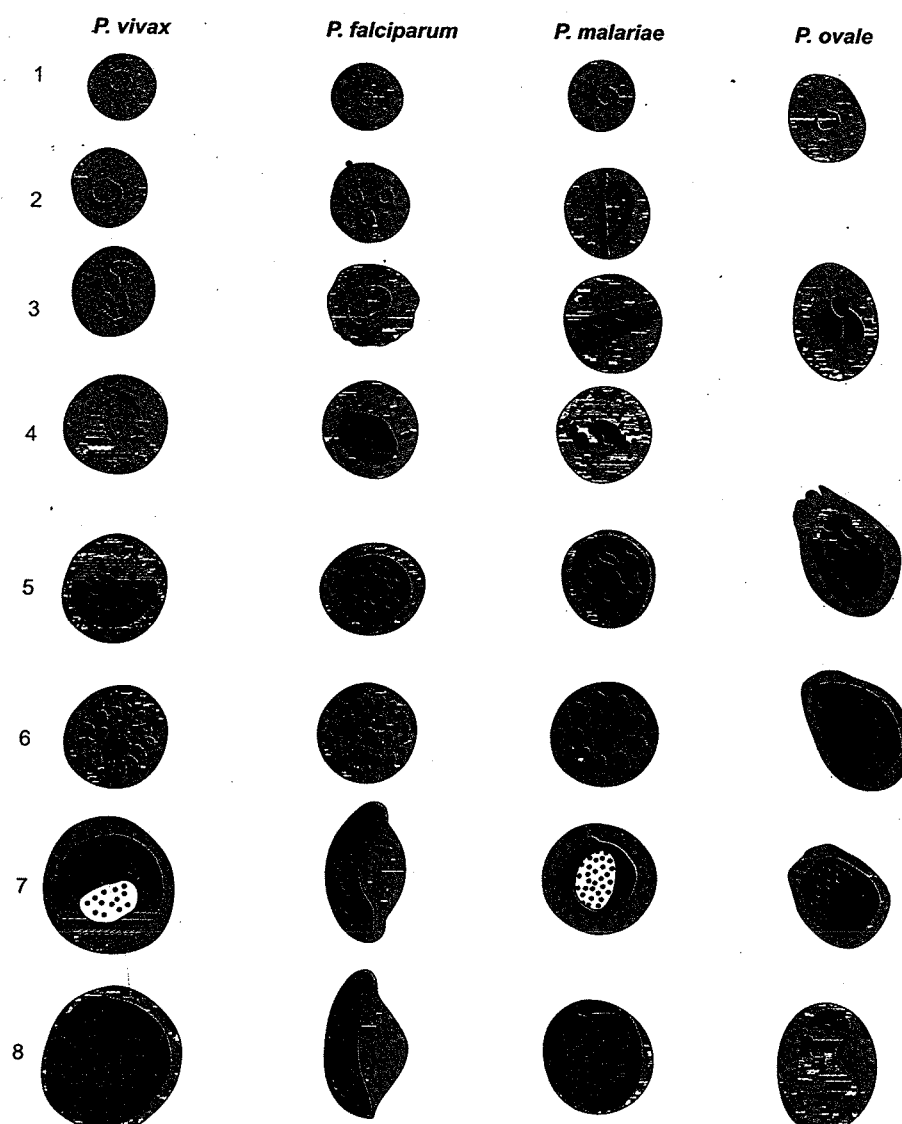
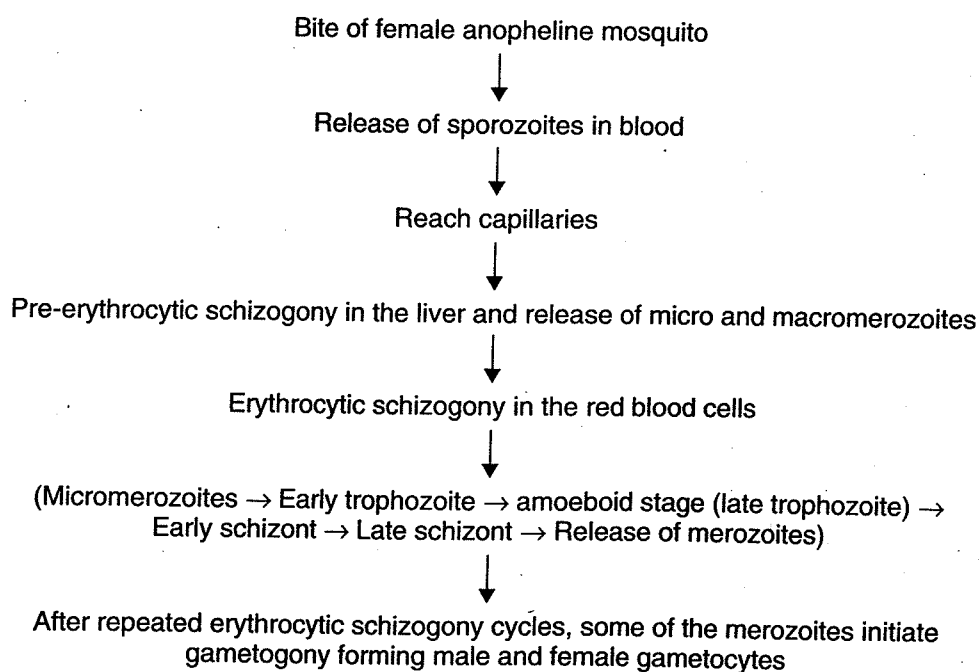


Fig. 79.1 Morphology of malarial parasites: 1 ring stage – early trophozoite, 2 late trophozoite, 3 amoeboid form, 4 early schizont, 5 late schizont, 6 mature schizont with merozoites, 7 male gametocytes, 8 female gametocytes.



Flowchart 79.1 Lifecycle of malarial parasite in human body

- Sporozoites in liver cells change to large, round bodies and undergo repeated division to form pre-erythrocytic schizont, which matures in 6–16 days and bursts releasing thousands of pear-shaped merozoites. These are of two types:
 1. micromerozoites – enter the circulation
 2. macromerozoites – re-enter the liver cells
- This cycle lasts for 7–8 days in *P. vivax*, 5–7 days in *P. falciparum*, 9 days in *P. ovale* and 14–16 days in *P. malariae*. No clinical illness or damage to liver cells is produced

2. Erythrocytic schizogony

- Micromerozoites released in circulation invade the RBCs by a process of endocytosis
- Inside the RBC, the merozoite becomes rounded body with a vacuole in the centre, peripheral cytoplasm and nucleus at one pole
- When stained with Romanowsky stain, the cytoplasm stains blue, nucleus – red and central vacuole—unstained. It appears like an annular or ring; hence it is called ring stage – **early trophozoite stage**. It further develops into amoeboid stage – it increases in size, becomes irregular in shape and shows amoeboid motility – **late trophozoite stage**
- When amoeboid stage reaches a certain stage of development and when its nucleus starts division, the stage is called **schizont**. Initially, nucleus divides to form small nuclei without the division of cytoplasm known as **early schizont**. Then each daughter nucleus is surrounded by cytoplasm. This stage is known as **late schizont**. The late schizont develops into **mature schizont** in which small merozoites are seen, each having a nucleus with surrounding cytoplasm
- The mature schizont bursts releasing the merozoites into circulation, which again invade the fresh RBCs and cycle is repeated. The rupture of the mature schizont releases large quantities of pyrogens responsible for clinical attack of malaria.
- This cycle lasts for 48 hours in *P. vivax*, *P. falciparum* and *P. ovale*, and 72 hours in *P. malariae*
- During this stage, the parasite feeds on the haemoglobin, which is not completely digested and leaves behind an iron containing residue – a haematin – globulin pigment known as malarial pigment

3. Gametogony

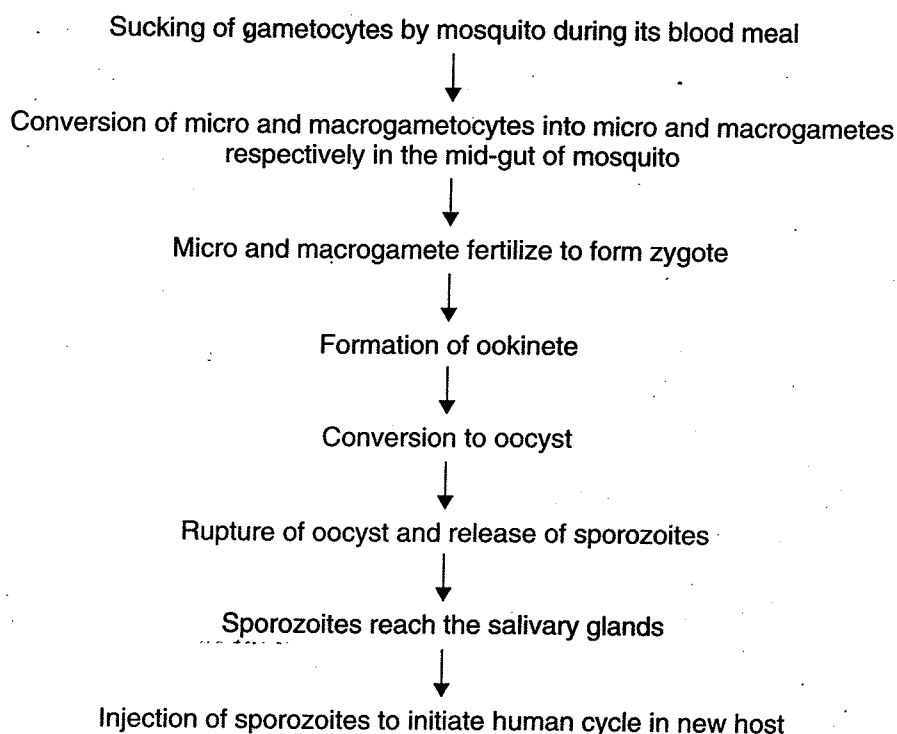
- After repeated cycles of erythrocytic schizogony, some merozoites develop into sexually differentiated forms called **gametocytes**. This process is known as **gametogony**, which takes place within the internal organs (spleen and bone marrow) and mature forms appear in peripheral circulation. Maturation is completed in about 4 days.
- The mature gametocytes are round in shape, except in *P. falciparum* in which they are crescent shaped
- The female (macrogametocyte) is larger than male (microgametocyte) and females are more in number
- They do not produce any febrile reaction and are produced for continuation of species and are taken up by mosquito. About 12 or more/mm³ of blood are required to make mosquito infective. The individuals who harbour gametocytes are known as **carriers**

4. Exoerythrocytic schizogony

- It is a local liver cycle that persists even after the establishment of blood infection. It is seen in *P. vivax*, *P. ovale* and probably in *P. malariae* but disappear in *P. falciparum*. It acts as a source of merozoites and causes relapses.

Mosquito Cycle (Flowchart 79.2)

- 1 Mosquito cycle starts with ingestion of gametocytes from infected person by female anopheline mosquito during its blood meal. Gametocytes reach the mid-gut where the



Flowchart 79.2 Lifecycle of malarial parasite in a mosquito.

microgametocyte divide to form 4–8 microgametes and macrogametocyte matures to form one macrogamete.

- One microgamete fertilizes macrogamete to produce a **zygote**, which lengthens and matures to form **ookinete**, which penetrates the gut wall and develops into **oocyst** in which **sporozoites** develop
- Oocyst matures, increases in size and finally ruptures releasing sporozoites, which migrate throughout the body and reach salivary glands and finally penetrate the acinar cells and enter the salivary ducts
- When such a mosquito bites, release sporozoites in skin capillaries to initiate human cycle

✓ Pathogenicity

- The disease caused is known as **malaria**. (Mala–bad and aria–air, derived from Italian words– as it was believed to be caused by foul emanations from the soil).
- The incubation period is generally 9–30 days:
 - Shortest in *P. falciparum*—12 days
 - Longest in *P. malariae*—28–30 days
 - In *P. vivax* and *P. ovale*—13–17 days
 - It may be 9 months with some *P. vivax* strains
- Each species causes a characteristic fever, designated as follows:

1. *Vivax malaria* (*benign tertian malaria*)

Caused by *P. vivax*. Fever recurs after intervals of 48 hours or every third day, hence tertian. It is less dangerous, hence benign.

2. *Falciparum malaria* (*malignant tertian malaria*)

Caused by *P. falciparum*. Fever recurs after intervals of 48 hours or every third day, hence tertian. It is dangerous, hence malignant. Also called as subtertian—because fever may recur before 48 hours.

3. *Malariae malaria* (quartan malaria)

Caused by *P. malariae*. Fever recurs after intervals of 72 hours or every fourth day.

4. *Ovale tertian malaria*

Caused by *P. ovale*. Fever recurs after intervals of 48 hours or every third day.

Clinical Features

The typical picture consists of periodic bouts of fever with chills, followed by anaemia and splenomegaly.

- **Febrile paroxysms:** Show three successive stages:
 - **The cold stage**—lasts for 15–60 minutes, characterized by intense cold and uncontrollable shivering
 - **The hot stage**—lasts for 2–6 hours, characterized by high grade fever, severe headache, nausea, vomiting and abdominal discomfort
 - **The sweating stage**—lasts for 2–3 hours, characterized profuse sweating
- **Anaemia:** Microcytic or normocytic, hypochromic type, more pronounced in *P. falciparum*
- **Splenomegaly:** Important physical sign, spleen is usually palpable by the second week

Recurrences in Malaria

It is of the following two types:

1. **Recrudescence:** In *P. falciparum*, following the development of immunity, the clinical attack subsides but the parasites are not completely eliminated and they persist in some RBCs. The number of these forms may increase in number and cause fresh attack of malaria within a year or two. This is known as recrudescence
2. **Relapse:** In *P. vivax* and *P. ovale* some sporozoites on entering the hepatocytes remain dormant in a cryptobiotic phase called **hypnozoites** (sleeping sporozoites). These forms are activated from time to time to form merozoites, which infect RBCs and cause recurrence of malaria. It is known as relapse.

Complications of *P. falciparum* Infection

- **Pernicious malaria (malignant malaria):** It is a life-threatening complication of *P. falciparum* infection, if infection is not effectively treated. Clinically it may present as:
 - **Cerebral malaria**—characterized by hyperpyrexia, convulsion, coma and paralysis
 - **Algid malaria**—characterized by cold and clammy skin, peripheral circulatory failure, hypotension, and gastrointestinal symptoms
 - **Septicaemic malaria**—characterized by high grade fever, bilious remittent fever, pneumonia and cardiac syncope
- **Black water fever:**
 - It occurs as a result of massive intravascular haemolysis caused by anti-erythrocyte antibodies
 - Characterized by haemoglobulinaemia and haemoglobinuria—passage of dark red or blackish urine; hence the name black water
 - Other symptoms are fever and rigour, bilious vomiting and prostration, circulatory collapse and acute renal failure
 - Chloroquine is the drug of choice for treatment

Laboratory Diagnosis

Specimens: Blood, a finger prick sample is sufficient. It should be collected before the administration of antimalarial agents. The parasites are abundant during febrile paroxysm—a few hours

after the peak of fever. Blood sample collected during this period gives positive results. Rarely, aspirates or material obtained by sternal puncture.

- **Direct microscopy:** Demonstration of malarial parasite in peripheral blood smear (PBS) in thick film—allows quick detection and thin film—for species identification. The blood smear is stained by **Leishman's stain**, **Giemsa stain**, **Wrights' stain** or **Field's stain**. In India, **JSB stain** (Singh and Bhattacharji)—rapid method—is commonly used. The smear is observed under oil immersion objective and at least 200 fields should be observed before it is declared negative
- **Quantitation of parasites** – PBS is graded as follows:
 - + = 1–10 parasites per 100 thick film fields
 - ++ = 11–100 parasites per 100 thick film fields
 - +++ = 1–10 parasites per single thick film field
 - ++++ = more than 10 parasites per single thick film field
- **Use of PBS examination**
 - To find out the percentage of parasitized RBCs
 - To find out species of malarial parasite
 - To detect malarial pigment that gives clue regarding the infection in absence of malarial parasite

Other Methods

1. **Fluorescent staining method:** Fluorochromes such as acridine orange, which stains nuclear DNA green in colour and cytoplasmic RNA red in colour that helps in the identification of the malarial parasites. This method can be used for rapid staining and rapid screening of smears
2. **Quantitative buffy coat (QBC) method:** Acridine orange is useful to identify malarial parasite by the quantitative buffy coat method
3. **Detection of antigen** - Dipstick test is an enzyme immunoassay test used for detection of *P. falciparum* antigen (histidine-rich protein II – PfHRP2) in the serum, test detecting parasitic lactate dehydrogenase, ELISA and RIA.

Serological tests: Different serological tests are used to demonstrate specific antibodies. These tests include:

- Immunoprecipitation
- Complement fixation test
- Indirect haemagglutination test
- ELISA
- Flocculation test
- Counter current immunoelectrophoresis
- Indirect immunofluorescence test
- RIA

Nucleic acid Detection

By using DNA and RNA probes and PCR.

■ Write in short the treatment for malaria.

- Chloroquine: Drug of choice
- In chloroquine resistant *P. falciparum*: A combination of sulphadoxine and pyrimethamine
- Primaquine: Acts on exo-erythrocytic parasites—hypnozoitocidal drug, used in relapse of *Plasmodium vivax*

80

Chapter

Other Medically Important Protozoan Parasites

Discuss in brief the morphology, lifecycle, pathogenicity and laboratory diagnosis of *Toxoplasma gondii*.

Morphology

It occurs in three forms—trophozoite, tissue cyst and oocyst (Fig. 80.1). All the three forms occur in definitive host—cat and other felines, while only asexual forms—trophozoites and cysts occur in intermediate host—humans, other mammals and birds.

1. Trophozoite stage (Fig. 80.1a)

- Size: $3 \times 7 \mu$
- Shape: Crescent-shaped—one end pointed and the other end rounded
- Nucleus: Ovoid, situated near blunt end, red in colour
- Cytoplasm: Azure blue when stained with Giemsa stain
- Multiplication: Endodyogeny or internal budding

2. Tissue cyst (Fig. 80.1b)

- Size: $200 - 1000 \mu$
- Shape: Round or oval
- Surrounded by a thick cyst wall
- Contains large number of slowly multiplying bradyzoites
- Found particularly in brain and muscles
- Cat acquires infection, when it feeds on mice containing tissue cysts

3. Oocyst (Fig. 80.1c)

- Size: $10-15 \mu \times 8-12 \mu$
- Shape: Spherical or ovoid
- Contains a sporoblast—with two sporocysts containing 8 sporozoites
- This stage occurs only in intestine of definitive host—the cat. They can infect cats and intermediate hosts.

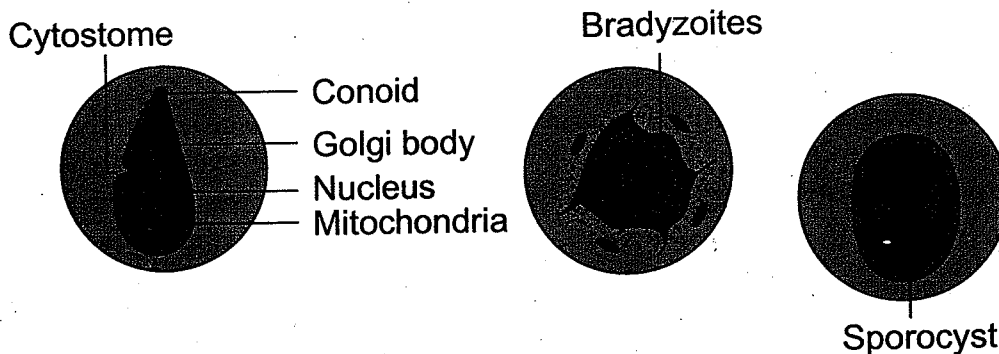


Fig. 80.1 *Toxoplasma gondii*: (a) trophozoite (b) cyst and (c) oocyst.

✓ Lifecycle

Definitive host: Cat and other felines—all the three forms occur in definitive host.

Intermediate host: Human beings, other mammals and birds—only asexual forms (trophozoites and cysts) occur in intermediate host. The lifecycle can be divided into two phases: exoenteric cycle and enteric cycle.

Lifecycle is complex – three different interlinked cycles:

- First – feline cycle
- Second – feline – nonfeline cycle
- Third – nonfeline – nonfeline cycle

1. Exoenteric cycle

- Occurs in intermediate host. Infection occurs by ingestion of mature oocyst in soil or water by intermediate host
- Oocyst releases sporozoites that infect the intestinal epithelial cells.
- Sporozoites multiply in the host cell forming endozoites (also called tachyzoites or trophozoites)
- When number increases, host cell ruptures releasing trophozoites, which infect other cells and multiply
- **During acute stage** of infection, the proliferating trophozoite inside the host cell may appear rounded and enclosed by the host cell membrane—appear like a cyst and is called **pseudocyst** or **colony**, which is differentiated from true cyst by its staining reaction—true cyst is not stained by silver. Trophozoites spread to various organs and tissues through blood and lymph.
- **During chronic phase**, the multiplying parasite produces a cyst wall and gets converted into cyst. This occurs in the muscles and various other tissues and organs. Cyst remains viable in tissues for several years. It produces infection when raw or undercooked meat is eaten. In the intestine, cyst wall is disrupted by peptic or tryptic digestion and parasites are released, which invade epithelial cells of intestine and reach various tissues and organs through blood and lymph.

2. Enteric cycle

- Occurs in definitive host. Cat is infected by ingestion of tissue cyst or oocysts
- Parasite develops in intestinal epithelial cells—schizogony and gametogony take place. Male and female gametocytes are produced during gametogony, which fertilize to form the zygote that finally becomes oocyst.
- The oocyst passed in faeces is not infectious. It develops in soil or water forming two sporocysts from a single sporoblast
- Each sporocyst develops four sporozoites, thus a mature oocyst contains 8 sporozoites. It is infective when ingested; it releases sporozoites in the intestine, which initiate infection

✓ Pathogenicity

The disease caused is known as toxoplasmosis.

✓ Clinical Features

Most human infections—asymptomatic. Clinically, occurs in 2 forms—congenital and acquired

Congenital toxoplasmosis

- Transmission—transplacental
- Most infected newborns—asymptomatic
- Some develop symptoms after weeks, months or years after birth
- The symptoms are chorioretinitis, strabismus, blindness, deafness, epilepsy or mental retardation
- A few are born with symptoms of **acute toxoplasmosis** characterized by fever, jaundice, diarrhoea, petechial rashes, hydrocephalus, microcephaly, cerebral calcification, micro-ophthalmia,

cataract, glaucoma, chorioretinitis, optic atrophy, lymphadenitis, pneumonitis, myocarditis and hepatosplenomegaly

Acquired toxoplasmosis

- Acquired by ingestion of milk, meat, egg or by inhalation or by inoculation through skin cracks and small abrasion
- In immunocompetent individuals—unrecognized in 90%
- The acute symptoms are lymphadenopathy (cervical lymph nodes are commonly involved), fever, headache, myalgia, and splenomegaly
- In immunocompromised host, severe illness is seen. It may be in the form of:
 - Atypical pneumonitis
 - Myocarditis
 - Meningoencephalitis
 - Cerebral toxoplasmosis
 - Generalized lymphadenopathy

Laboratory Diagnosis

It includes:

- **Microscopic demonstration** of trophozoites by Giemsa stain in smears of bone marrow, splenic puncture, cerebrospinal fluid and cysts in tissue sections
- **Animal inoculation:** The specimen is inoculated in mice, guinea pigs or hamsters to demonstrate trophozoites after 7–10 days. Serum may show antibodies
- **Serological tests:** Different serological tests are used to demonstrate antibodies. These tests include:
 1. **Tests used for detection of IgM** are as follows:
 - (a) IgM – ELISA
 - (b) IgM – indirect fluorescent Ab test
 - (c) IgM – immunosorbent agglutination assay
 - Detection of IgM indicates recent infection. In pregnant women, it indicates an acute infection and risk of infection to the foetus. Its presence in neonate indicates congenital infection
 2. **Tests used for detection of IgG** are as follows:
 - (a) Complement fixation test
 - (b) Indirect haemagglutination test
 - (c) Indirect immunofluorescence test
 - (d) ELISA
 - (e) Sabin and Feldman dye test—uses alkaline methylene blue, which stains *T. gondii*. If patient's serum contains specific Abs, the staining of *T. gondii* is inhibited. The test is not widely used now-a-days because it requires live *T. gondii*
 - Demonstration of rising titre of IgG—important in confirmation of diagnosis. In pregnant women, rising titre indicates an acute infection and risk of infection to the foetus. Rising titre in neonates indicates acute congenital infection. In ocular toxoplasmosis, a positive IgG titre confirms the diagnosis

Write in short the treatment for toxoplasmosis.

- A combination of sulphadiazine and pyrimethamine
- Spiramycin can be used as an alternative to pyrimethamine in pregnant women

■ Discuss in brief the morphology, lifecycle, pathogenicity and laboratory diagnosis of *Balantidium coli*.

Morphology

It occurs in the following two forms:

1. **Trophozoite or vegetative form** (Fig. 80.2a)

- Shape: Oval
- Size: 60–70 μ \times 40–50 μ
- Motility: Motile with the help of cilia covered all over the body
- Nucleus: Two nuclei, one large, kidney shaped—**macronucleus**, one small, round—**micronucleus**
- Anterior end: Narrow, shows cytostome (mouth) and cytopharynx
- Posterior end: Broad, shows small pore (cytopyge—anus)
- Two contractile vacuoles: One in middle and other at posterior end
- Many food vacuoles, tissue debris, white blood cells and red blood cells are present in cytoplasm

2. **Cyst stage** (Fig. 80.2b)

- Shape: Oval
- Size: Smaller than trophozoite—50–60 μ in diameter
- Nucleus: Two nuclei—macronucleus and micronucleus
- Cyst wall: Thick, transparent, double-layered wall

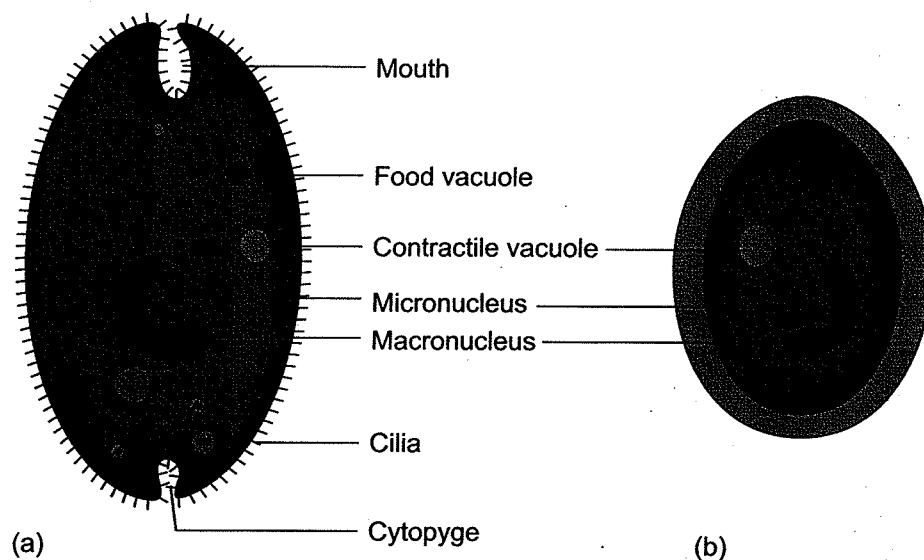


Fig. 80.2 *Balantidium coli*: (a) trophozoite and (b) cyst.

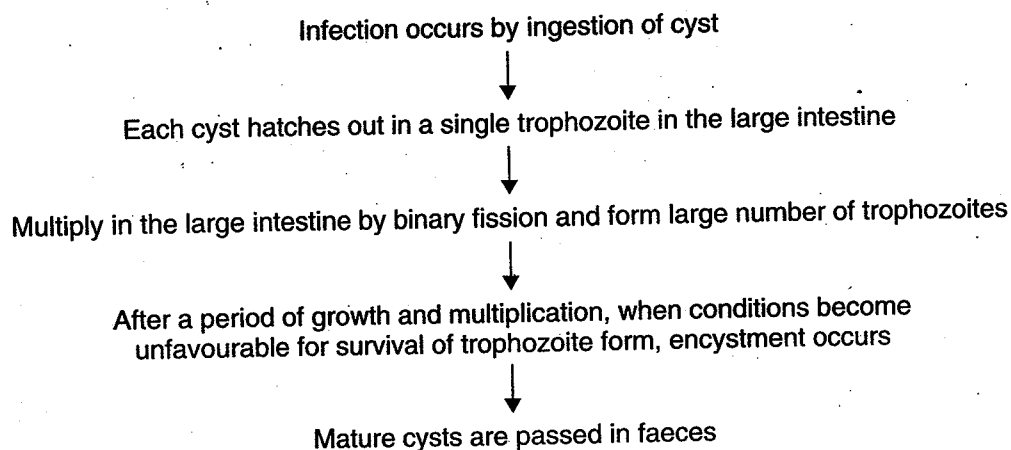
Lifecycle (Flowchart 80.1)

Definitive host: Pig is the natural host, man is accidental host

Intermediate host: Not required, passes lifecycle in one host

Pathogenicity

Clinical disease occurs when trophozoites invade intestinal mucosa.



Flowchart 80.1 Lifecycle of *Balantidium coli*.

Clinical Features

The disease caused is known as balantidiasis characterized by:

- Mucosal ulcers and submucosal abscess. Diarrhoea or dysentery with abdominal pain, tenesmus, nausea and vomiting
- Occasionally, intestinal perforation with peritonitis and involvement of genital and urinary tracts

Laboratory Diagnosis

Demonstration of parasite in stool sample by saline and iodine preparation.

■ Write in short the treatment for balantidiasis.

- Tetracycline
- Metronidazole
- Nitrimidazine

■ Discuss in brief *Cryptosporidium parvum*.

- *Cryptosporidium parvum*—First observed by Tyzzer (1907) in the stomach of laboratory mice. Recently, it has assumed importance as an important cause of diarrhoea in AIDS patients
- **Morphology**—Oocyst 2–5 μ in diameter, contain 4 sporozoites—infective form found in human faeces

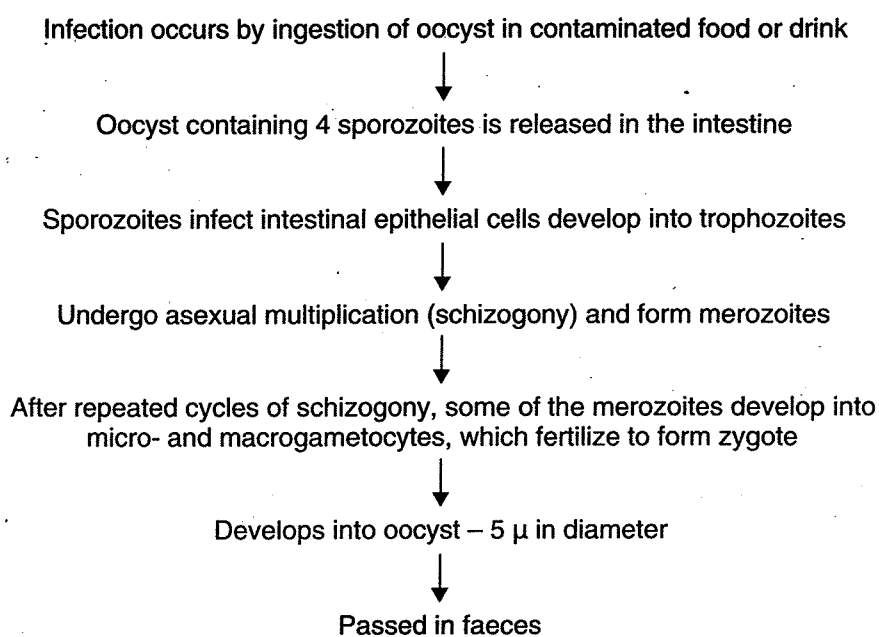
Lifecycle (Flowchart 80.2)

It completes its lifecycle (both sexual and asexual phases) in a single host

Clinical Features

The disease caused is known as cryptosporidiosis

- Infection may be asymptomatic or a self-limited febrile illness or watery diarrhoea in previously healthy individuals
- Profuse watery diarrhoea leading to severe dehydration, fever, profound weight loss and emaciation in AIDS patients and in immunodeficient individuals



Flowchart 80.2 Lifecycle of *Cryptosporidium parvum*.

Laboratory Diagnosis

- **Specimen:** Stool
- **Demonstration of oocysts** – in unstained wet preparation
 - Modified Ziehl–Neelsen stain—the internal structure of *Cryptosporidium parvum* appear acid fast. It helps to differentiate it from yeast
 - Nuclei of the sporozoites in the oocysts can be visualized by staining with diamidinophenylindole (DAPI)
- **Detection of Ag:** By direct immunofluorescence stain and ELISA
- **Detection of Abs:** By indirect immunofluorescence and ELISA

Treatment

No specific treatment. Tetracycline, metronidazole and nitrimidazine can be used.

■ Discuss in brief *Isospora belli*.

- *Isospora belli* is a coccidian parasite causing diarrhoea in human beings
- It resides in epithelial cells in small intestine, where schizogony and sporogony take place

Morphology

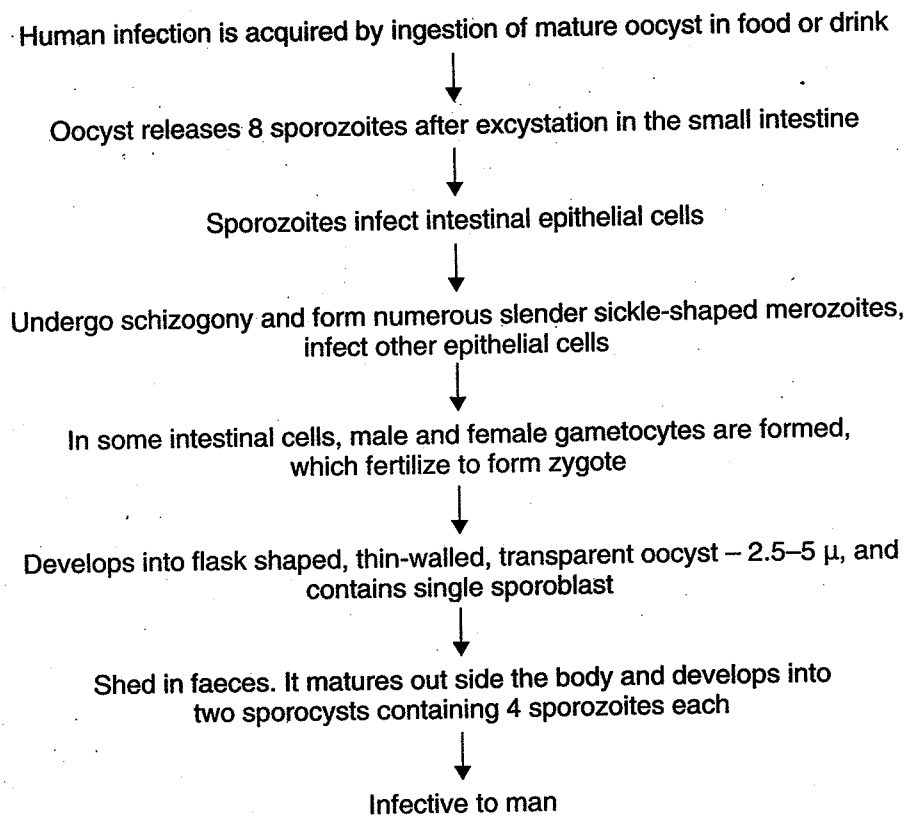
Oocysts are elliptical, $20\text{--}33\ \mu \times 10\text{--}19\ \mu$, surrounded by two-layered wall, contain two sporocysts each with 4 sporozoites. It is an infective form found in faeces

Lifecycle (Flowchart 80.3)

It completes lifecycle (both sexual and asexual phases) in one host.

Clinical Features

- Infection is usually asymptomatic
- Self-limited, but protracted watery diarrhoea, lasting for long duration, particularly in AIDS, immunodeficient and malnourished patients, leading to weight loss



Flowchart 80.3 Lifecycle of *Isospora belli*.

Laboratory Diagnosis

- Demonstration of oocysts in stool in unstained wet preparation
- Demonstration of oocysts by modified Ziehl-Neelsen stain
- Fluorescent auramine stain is also useful

Treatment

Trimethoprim plus sulfamethoxazole

81

Chapter

Helminths: Medically Important Intestinal Cestodes

LSN

State the common name, history, geographical distribution, habitat, morphology, lifecycle, pathogenicity and laboratory diagnosis of *Taenia saginata* (beef tapeworm) and *Taenia solium* (pork tapeworm).

Common name, history, geographical distribution, habitat and morphology and pathogenicity of *Taenia saginata* and *T. solium* are presented in Table 81.1.

Table 81.1 Characteristic features (common name, geographical distribution, habitat and morphology, pathogenicity) of *Taenia* spp.

	<i>T. saginata</i>	<i>T. solium</i>
Common name	Beef tapeworm or unarmed tape-worm	Pork tapeworm or armed tapeworm
History	Goeze in 1782 differentiated it from <i>T. Solium</i> , Leuckart in 1863 demonstrated cattle as an intermediate host	Discovered by Linnaeus in 1758, Beneden in 1854 described lifecycle, Kuchenmeister in 1855 demonstrated adult worm in the human intestine
Geographical Distribution	Worldwide <ul style="list-style-type: none"> • Common in Mohammedans • Not generally found amongst Hindus 	Worldwide <ul style="list-style-type: none"> • Uncommon in Mohammedans
Habitat	Small intestine (jejunum)	Small intestine (upper jejunum)
Morphology		
Adult worm	Tape-like 5–10 meter in length, white, semitransparent	Tape-like 2–3 meter in length, white, semitransparent
Scolex (Head)	<ul style="list-style-type: none"> • Large, quadrate with 4 suckers may be pigmented • Head without rostellum and hooklets (Fig. 81.1) 	<ul style="list-style-type: none"> • Small, globular with 4 sucker • Head provided with rostellum armed with double row of alternating large and small hooklets (Fig. 81.1)
Neck	Long, thin and fragile	Short and thick, 5–6 mm long
Proglottides (segments)		
Number	1000–2000	800–900
Gravid segments		
Size	20 × 5 mm	12 × 6 mm
Expelled	Singly	In chains of 5 or 6
Uterus	Central longitudinal stem with 15–30 lateral branches on each side	Central longitudinal stem with 5–10 compound lateral branches on each side
Common genital opening	In the posterior end on lateral margin of each segment	Mid-lateral

Continued

Table 81.1 Characteristic features (common name, geographical distribution, habitat and morphology, pathogenicity) of *Taenia* spp.—cont'd

	<i>T. saginata</i>	<i>T. solium</i>
Vaginal sphincter	Present	Absent
Ovary	Two without accessory lobes	Two with accessory lobes
Testes follicles	300–400 (Fig. 81.1)	150–200 (Fig. 81.1)
Life span	10 years	25 years
Eggs (Fig. 81.1)	Acid fast	Non-acid fast
Shape	Round/oval	Similar to <i>T. saginata</i>
Size	33–43 μ in diameter	
Colour	Brown (bile stained)	
Membranes	Two membranes: 1. Outer—thin and transparent 2. Inner—embryophore; it is thick, brown, radially striated, encloses embryo (oncosphere) Embryo—contains six hooklets (hexacanth embryo) Infective to cattle only	
Larvae (bladder worms or Cysticerci)	<i>Cysticercus bovis</i>	<i>Cysticercus cellulosae</i>
Size	5–10 mm \times 3–4 mm Found in muscles of cow, buffalo Not found in human beings Infective to human beings	8–10 mm \times 5 mm Found in muscles of pig Found in human beings Infective to human beings
Pathogenicity—intestinal taeniasis (tapeworm infection)	Characterized by abdominal discomfort, chronic indigestion, anaemia. Crawling segments rupture around perianal area Cysticercosis does not occur	Similar to <i>T. saginata</i> but less conspicuous Cysticercosis • Fatal systemic condition • Cysticerci are found in muscle and subcutaneous tissue, eyes, brain

Lifecycle (Flowchart 81.1)

It needs two hosts to complete its lifecycle

Definitive host: Human beings

Intermediate host: Cow, buffalo in *T. saginata*. Pig or rarely humans in *T. solium*

When human being acts as intermediate host, cysticerci develop in various parts further not taken away by any definitive host, thus the lifecycle of parasite comes to an end in *T. solium*.

Laboratory Diagnosis

This includes:

- Demonstration of eggs and segments of worms in faeces
- Demonstration of eggs in anal swab collected by using NIH swab in *T. saginata*
- Demonstration of *Taenia* Ag in faeces by ELISA
- Demonstration antibodies by indirect haemagglutination test, indirect immunofluorescence test and ELISA—has limited value in intestinal taeniasis

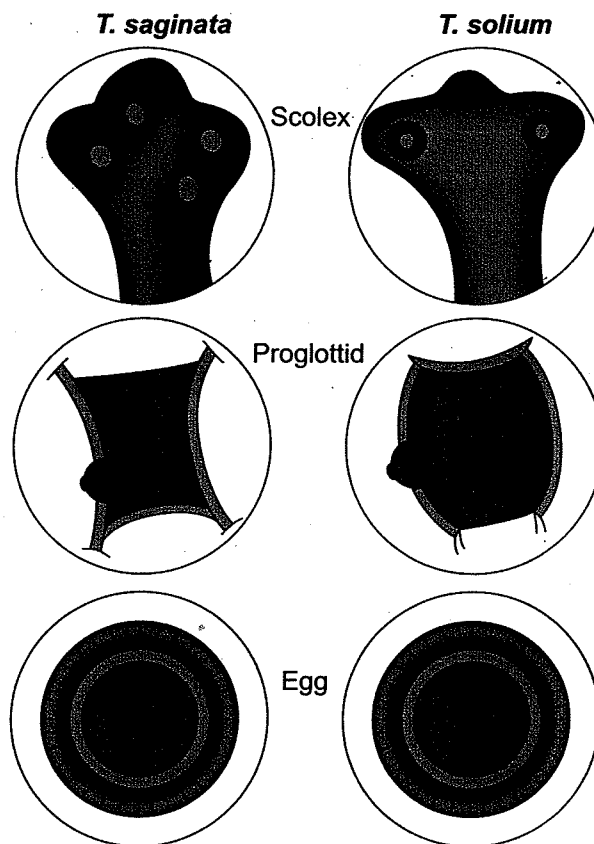
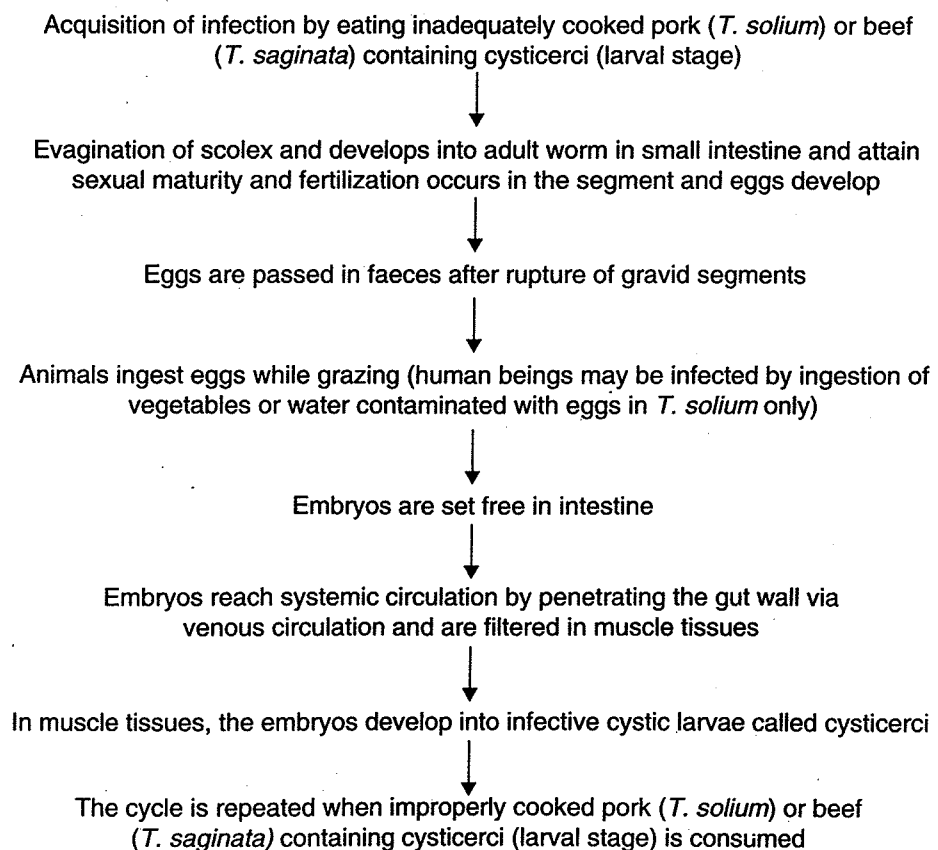


Fig. 81.1 Scolex, proglottides and eggs of *Taenia saginata* and *Taenia solium*.



Flowchart 81.1 Lifecycle of *Taenia* spp.

- Molecular diagnosis by DNA probes and polymerase chain reaction (PCR)
- Diagnosis of Neurocysticercosis by detecting antibodies by ELISA and immunoblot in CSF
- Antigen detection in CSF—by ELISA
- Histopathological diagnosis—demonstration of cysticerci in tissue biopsy
- Radiological diagnosis—X-ray, CT scan and MRI to demonstrate calcified cyst

■ **Write in short the treatment for taeniasis.**

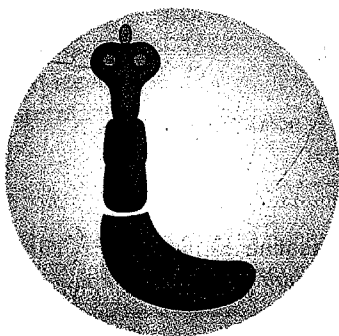
- Praziquantel is the drug of choice – safe and effective in a single oral dose – kills worms
- Niclosamide is also highly effective in a single oral dose and safe – kills the scolex and the anterior segment of the worm. Albendazole can also be used

LSN ■ **Discuss in brief the morphology, lifecycle, pathogenicity and laboratory diagnosis of *Echinococcus granulosus*.**

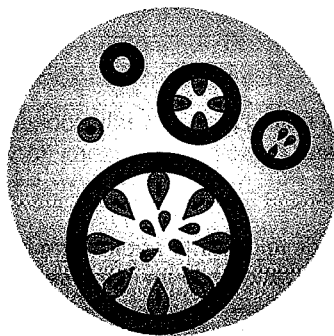
Morphology

Adult Worm (Fig. 81.2a)

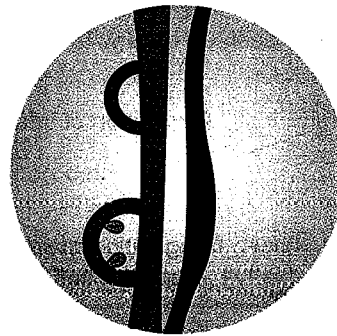
- It is a small tapeworm
- Size: Length 3–6 mm, it has head, neck and body
- Scolex: Pyriform, has four suckers with two rows of hooks
- Neck: It is short and thick
- Strobila or body: It consists of three segments: First—immature, second—mature and third—gravid
- Life span of adult worm is 6 months and hydatid cyst lasts for several years



a. Adult worm



b. Hydatid sand



c. Hydatid cyst wall

Fig. 81.2 *Echinococcus granulosus*: (a) adult worm, (b) hydatid cyst and (c) hydatid cyst wall.

Egg

- Eggs: Similar to *Taenia* spp
- Shape: Ovoid
- Size: 32–36 μ \times 25–32 μ
- Colour: Brown (bile stained)
- Outer layer surrounds inner embryophore
- Embryo contains 6 hooklets (hexacanth embryo)
- Eggs are infective to humans, cattle and sheep
- Excreted in faeces of canine animals
- From one egg - one larvae containing many solices from which number of adult worms are formed

Larval Form (Fig. 81.2b and c)

- It is hydatid cyst, which develops in tissue of intermediate host
- Found in various organs of humans and intermediate hosts
- It represents scolex of future adult worm and remains invaginated within vesicular body
- Hydatid cyst in humans is:
 - Typically unilocular, subspherical in shape and is filled with fluid
 - Growth rate is 1–5 cm/year. The cyst wall secreted by embryo has two layers:
 - Outer—cuticular layer (ectocyst)**—laminated hyaline membrane, appears as white of hard-boiled egg, 1 mm thick, elastic, when ruptures or incised curls on itself
 - Inner—germinal layer (endocyst)**—It is cellular, nucleate and thin, 22–25 micron. It is a vital layer of cyst and gives rise to:
 - Brood capsules with scolices
 - Specific hydatid fluid
 - Outer layer
- In few cysts, brood capsules fail to develop or even if they develop they do not contain scolices, those cysts are called **acephalocysts**
- **Hydatid fluid:** Characteristics are as follows:
 - Colourless, clear fluid
 - Specific gravity 1.005–1.010
 - Acidic pH 6.7
 - It is antigenic
 - It is highly toxic
 - Contains salts (sodium chloride, sodium sulphate and salts of succinic acid)
 - Hydatid sand—a granular deposit at the bottom contains liberated brood capsules, free scolices and loose hooklets

✓ **Lifecycle (Flowchart 81.2)**

It needs two hosts to complete its lifecycle. The natural lifecycle is completed between sheep and dog.

Definitive host: Dog (also fox, wolf and jackals)

Intermediate host: Sheep, cattle, pigs, goat, horse and human beings are accidental host

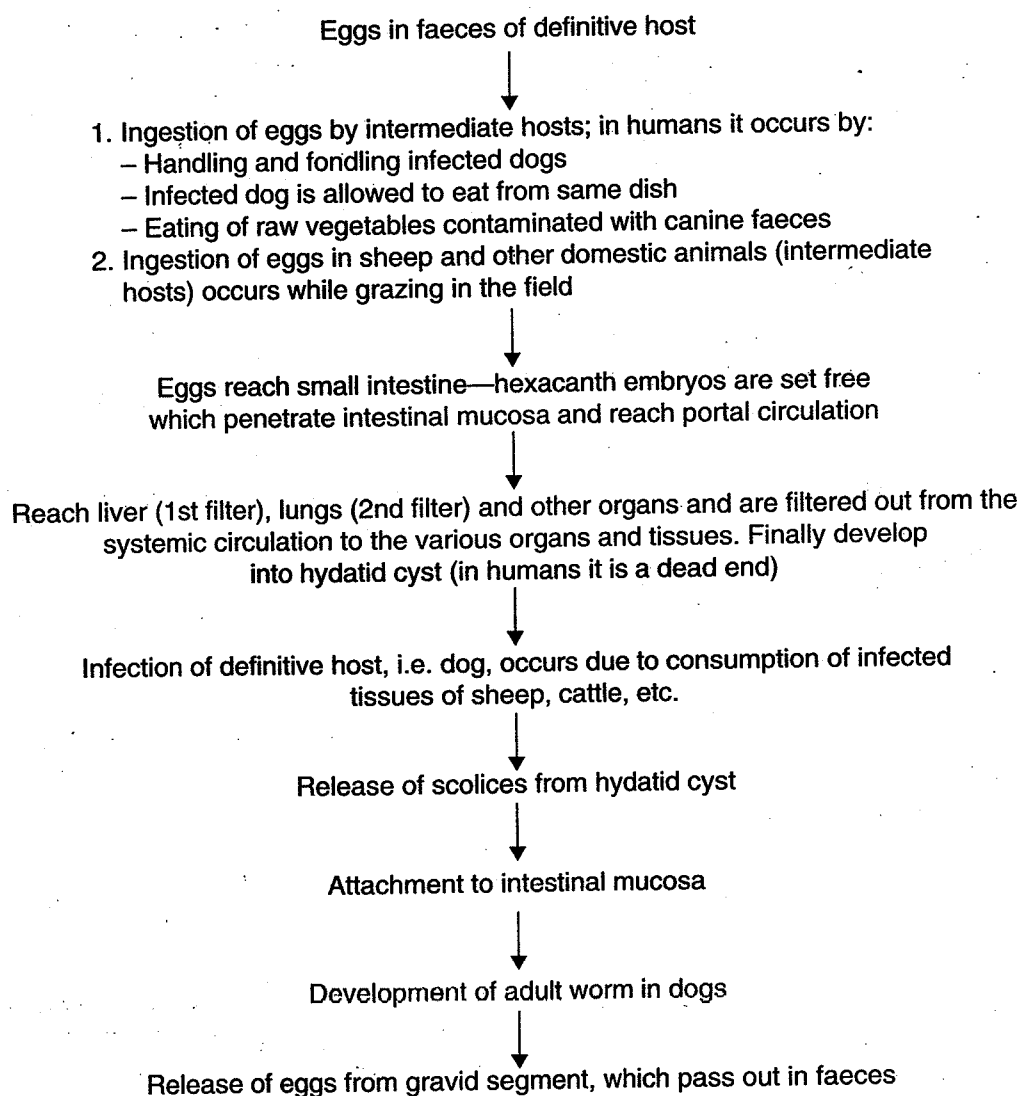
Pathogenicity

- The disease caused is known as unilocular hydatid disease
- It is characterized by the following features:
 - Hydatid cyst in subcutaneous tissue may be visible and palpable
 - Its presence can be detected at autopsy or by its pressure effects on the surrounding tissue or suppuration of cyst or rupture of cyst producing anaphylactic symptoms along with secondary echinococcosis localized or generalized
 - Organs involved - liver is the commonest site, followed by lungs and other organs
 - Usually one organ is involved but may be found in multiple organs
 - Liver hydatid cyst—may manifest with pain and swelling, nausea, vomiting
 - Lung hydatid cyst—presents with cough and chest pain
 - Hydatid cyst may rupture spontaneously and degenerate because of lack of nutrition

✓ **Laboratory Diagnosis**

The diagnosis includes:

- **Casoni's Test (skin test)**—0.2 ml of sterile hydatid fluid is injected in one arm—development of large wheel of 5 cm diameter with multiple pseudopodia in 30 minutes indicates positive reaction, which fades away in 60 minutes. Presently not in use



Flowchart 81.2 Lifecycle of *Echinococcus granulosus*.

- **Ag detection test:** Ag detection in serum can be done by ELISA, counter current immunoelectrophoresis, coagglutination test and latex agglutination test
- **Detection of Ab:** By indirect haemagglutination test, fluorescent antibody test, counter current immunoelectrophoresis, latex agglutination test, ELISA and Western blot test. They are of low sensitivity and low specificity and cannot differentiate old and new infection
- **Radiological diagnosis:** Plain X-ray, ultrasonography, CT scan, magnetic imaging resonance (MRI) can be used for diagnosis
- **Histopathological diagnosis:** Cyst wall can be demonstrated
- **Demonstration of protoscolices** in hydatid fluid by wet mount examination - Diagnostic aspiration is not recommended due to danger of anaphylaxis and secondary echinococcosis.

■ **Write in short the treatment for hydatid disease.**

- **Surgical:** Surgical removal of cyst—it is the major line of treatment
- **Medical:** Albendazole, mebendazole and praziquantel may be used

- ✓ ■ Discuss in brief the morphology, lifecycle, pathogenicity and laboratory diagnosis of *Hymenolepis nana* (dwarf tapeworm).

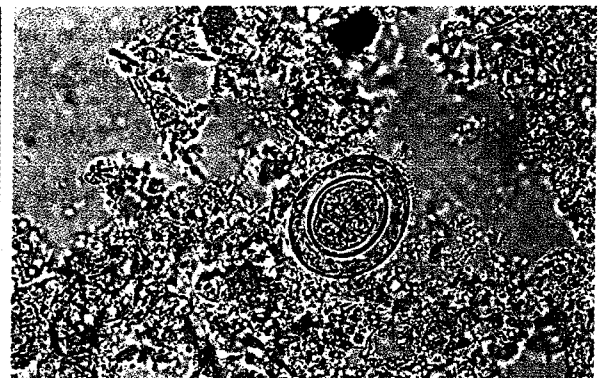
Morphology

Adult Worm (Fig. 81.3a)

- Smallest cestode infecting humans, measures 10–40 mm in length and 1 mm in breadth
- It has scolex, neck and proglottides
- Scolex: Globular, four suckers and rostellum remains invaginated in the apex with single row of hooklets. Neck: Long and segments
- Proglottides–number: 200 and size: 0.3×0.9 mm with marginal common genital opening on same side in each segment
- Life span: 2 weeks



(a)



(b)

Fig. 81.3 *Hymenolepis nana*: (a) scolex and (b) egg. (Source: Fig. 81.3(A) *Tropical Infectious Diseases: Principles, Pathogens and Practice*, Pages 839–847, Saunders, 2011. 81.3(B) (Source: *Journal of Medical Microbiology*, Fig. 86–10, 2005.)

✓ **Egg (Fig. 81.3b)**

- Shape: Spherical or oval
- Size: 30–45 μm in diameter
- Colourless
- It has two distinct membranes outer thin and inner embryophore enclosing embryo with three pairs of hooklets. Clear space is present in between two membranes and is filled with yolk granules and 4–8 polar filaments arising from poles of embryophore
- Infective to man
- It floats in saturated salt solution

Lifecycle (Flowchart 81.3)

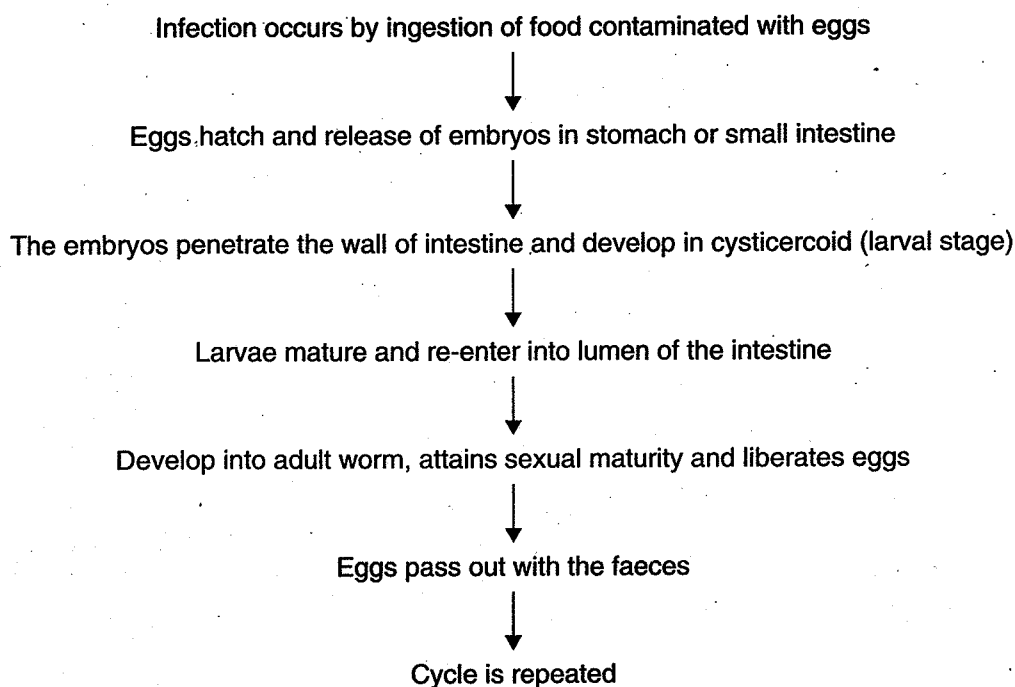
Simple, needs only one host to complete cycle

Definitive host: Human beings, rat and mouse serve as definitive as well as intermediate hosts.

Intermediate host: Not required

Autoinfection

Egg may develop again into larvae at the lower level of intestine and may cause infection in same host (autoinfection). *H. nana* is one of the exception to the general rule that the helminths do not multiply inside the body of definitive host.



Flowchart 81.3 Lifecycle of *Hymenolepis nana*.

✓ Pathogenicity

The disease caused is known as hymenolepiasis or hymenolipidosis. It is usually asymptomatic, but in heavy infection it may manifest as abdominal pain, and diarrhoea.

Laboratory Diagnosis

It includes:

- Demonstration of typical eggs in stool sample
- Demonstration of typical eggs in concentrated stool—by salt flotation technique

■ Write in short the treatment for hymenolepiasis.

Praziquantel, niclosamide and paromomycin

82

Chapter

Medically Important Trematodes

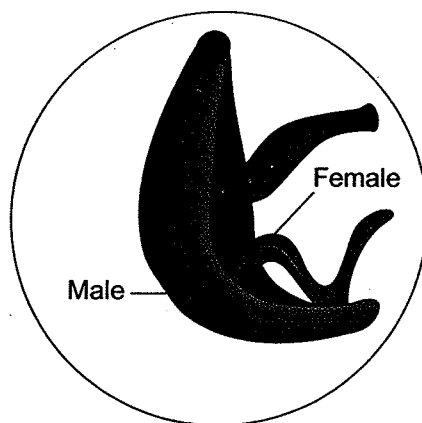
- Describe the morphology, lifecycle, pathogenicity and laboratory diagnosis of *S. haematobium* (the vesical blood fluke).

Morphology

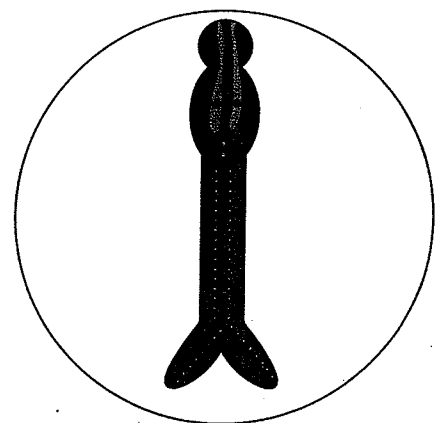
Adult worm (Fig. 82.1a)

- **Female worm**

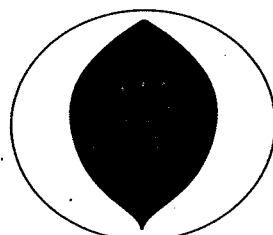
- It is cylindrical, longer, slender than males
- It is about 2 cm × 0.25 mm
- Its ovary is situated posterior to middle of the body
- It has two suckers – ventral and oral
- It lives in gynaecophoric canal of male
- Its uterus contains 20–30 eggs
- Fertilized female lays about 20–200 eggs per day during capulatory phase



a

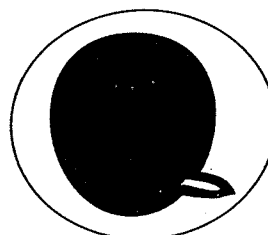


b

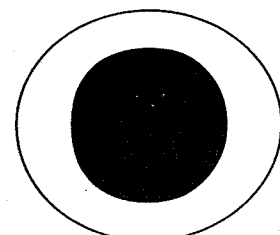


S. haematobium

c



S. mansoni



S. japonicum

Fig. 82.1 Schistosomes: (a) adult worm, (b) larva and (c) eggs.

- **Male worm**

- It is short and stout about 1–1.5 cm × 0.9 mm and broader than female
- It holds the female worm in gynaecophoric canal
- Its body surface is finely tubercular
- It has 4–6 testes in clusters

Eggs (Fig. 82.1c)

- Shape: Elongated and oval
- Size: 110–170 μ × 40–70 μ
- Colour: Yellow-brown
- It is nonoperculated and has terminal spine at posterior end
- Contains fully developed embryo called miracidium
- Eggs pass from venules to urinary bladder

Cercariae – Larval form (Fig. 82.1b)

- Shape: Elongated and oval
- Size: 150 × 60 μ
- It has two suckers and forked tail as long as body
- Body is curved with minute spike-like projections
- It is short-lived—24–72 hours
- It develops in second generation sporocysts

Lifecycle (Flowchart 82.1)

Completed in two hosts

Definitive host: Human beings

Intermediate host: Freshwater snail of genus *Bulinus*

Pathogenicity and Clinical Features

The disease caused is known as urinary schistosomiasis, bilharziasis, endemic haematuria or schistosomiasis haematobia.

Symptoms due to Cercariae

Allergic dermatitis: At the site of entry leading to intense irritation and papular pruritic rash—is also known as swimmer's itch.

Symptoms due to Eggs

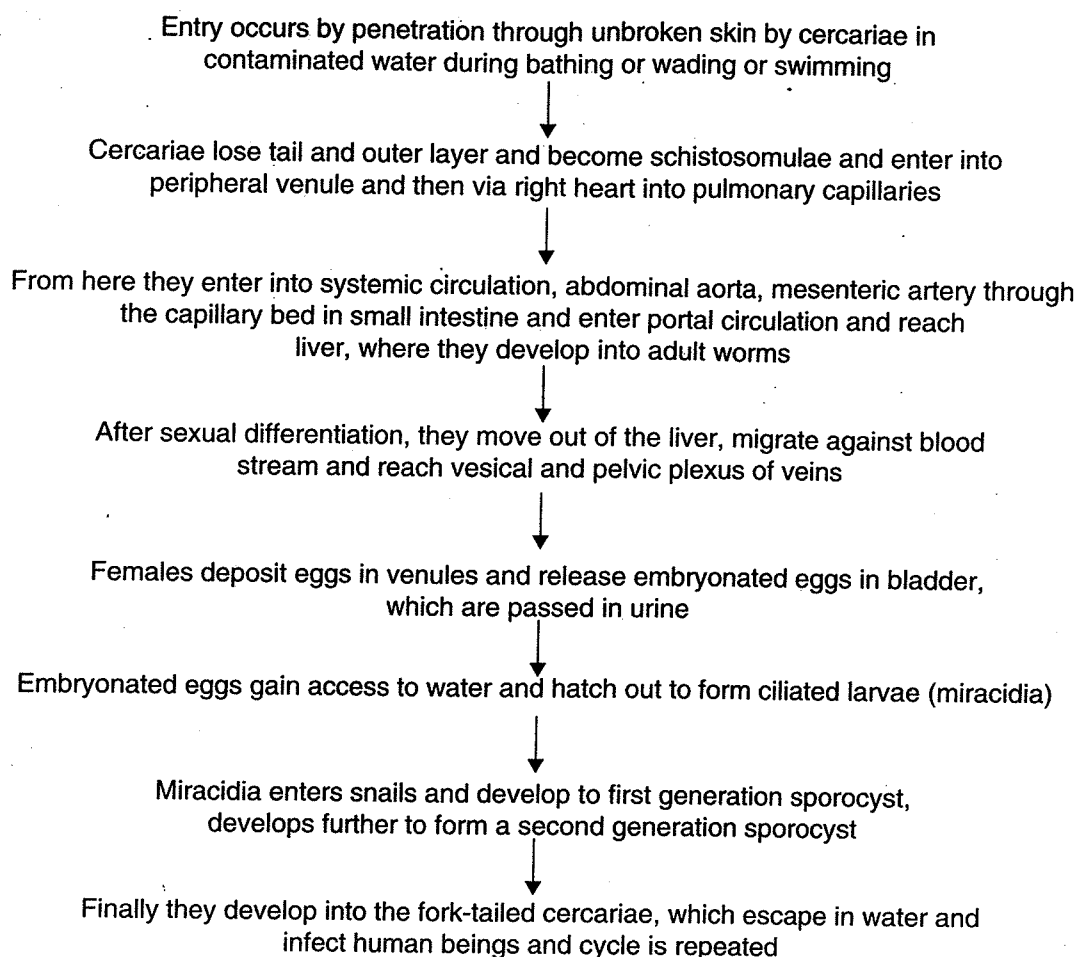
- **Egg granuloma:** It is delayed hypersensitivity reaction around egg; usually found in ureter and bladder.
- **Haematuria:** Eggs cause traumatic lesions leading to painless terminal haematuria, frequent micturition and dysuria.

Other symptoms

These are seen during circulation and maturation of *S. haematobium* in blood vessels. Toxic metabolites released during growth of schistosomulae in portal blood of the liver cause generalized anaphylactic reaction characterized by fever, urticaria, enlargement of liver and spleen called **Katayama fever** in Japan.

Complications

- Hydroureter, hydronephrosis, secondary infections and uremia may lead to renal failure
- Ectopic lesions in liver, lungs, intestine and central nervous system due to deposition of eggs
- Urinary bladder carcinoma due to chronic infection



Flowchart 82.1 Lifecycle of *Schistosoma haematobium*.

Laboratory Diagnosis

It includes:

- Demonstration of the typical eggs in urine collected around midday and biopsy of lesion in bladder
- Demonstration of the typical eggs in concentrated urine specimen—by centrifugation or filtration
- Egg counting—in 24 hours urine—to find out the severity of infection—more than 50 eggs per 10 ml of urine indicate heavy infection and less than 50 eggs indicate light infection
- Demonstration of Abs by indirect haemagglutination test, indirect immunofluorescence test, ELISA, RIA and immunoblot test is useful in ectopic schistosomiasis, patient in prepatent period, chronic cases and for epidemiological surveys
- Demonstration of Ag in serum and urine by CIEP, ELISA and RIA. Demonstration of Ag helps in detecting acute stage of infection. It also has prognostic value
- Demonstration of the typical eggs in vesical mucosal pieces crushed between two slides by histopathological examination
- Skin test—The intradermal allergic test – Fairley's test – can be used

■ Write in short the treatment for urinary schistosomiasis.

- Praziquantel is the drug of choice – single dose.
- Metrifonate can also be used as a cheaper alternative

■ **Describe the morphology, lifecycle, pathogenicity and laboratory diagnosis of *S. mansoni* (Manson's blood fluke).**

Morphology

Morphologically similar to *S. haematobium* except for the following features:

Adult worm

• **Female worm**

- It is about 1.4 cm × 0.25 mm
- Its ovary is situated anterior to middle of the body
- Its uterus contains 1–4 eggs
- Fertilized female lays about 100–300 laterally spine eggs per day

• **Male worm**

- It is about 1 cm × 1 mm
- Its body surface is grossly tubercular
- It has 6–9 testes in zigzag row

Eggs (Fig. 82.1c)

- Shape: Elongated and oval
- Size: 150 μ × 60 μ
- It has lateral spine more near rounded posterior end

Lifecycle

Needs two hosts to complete lifecycle

Definitive host: Human beings

Intermediate host: Fresh water snail of genus *Biomphalaria*

Lifecycle is similar to *S. haematobium* except for the site of localization. They migrate to radicals of inferior mesenteric vein to reach the sigmoidorectal venous plexus where they lay eggs, which finally excreted in faeces. The eggs on contact with water hatch out to miracidium, which penetrate intermediate host.

Pathogenicity and Clinical Features

- The disease caused is known as **schistosomiasis mansoni**; it is also known as **intestinal bilharziasis or schistosomal dysentery**
- The disease may present as:
 - **Acute infection**—Manifested as attack of dysentery characterized by abdominal pain, diarrhoea or dysentery
 - **Cercarial dermatitis**—Transient dermatitis at the site of entry
 - **Systemic illness**—Fever, malaise, abdominal pain, liver tenderness due to migrating worms
 - **Chronic infection leads to:**
 - Egg granulomas**—observed in the walls of colon and rectum leading to fibrosis, thickening of wall, papillomatous growth along entire length occurs
 - Ectopic lesions**—lead to splenomegaly, portal hypertension and hepatomegaly

Complications

Periportal fibrosis, gastrointestinal haemorrhage, cor pulmonale and transverse myelitis.

Laboratory Diagnosis

It includes:

- Demonstration of the typical eggs in faeces in acute cases
- Demonstration of the typical eggs in concentrated specimen in chronic cases in which number of egg is scanty. Sedimentation in 0.5% glycerinated saline or by acid ether method is used for concentration of faeces
- Demonstration of Abs by latex agglutination, ELISA, RIA and CIEP. Importance of these tests is like *S. haematobium*
- Demonstration of Ag in serum by CIEP and ELISA. The test is useful during acute or end stage disease when excretion of egg is minimal
- Demonstration of the typical eggs in rectal biopsy

■ **Write in short the treatment for *Schistosomiasis mansoni*.**

Treatment is same as mentioned for *S. haematobium* infection.

■ **Describe the morphology, lifecycle, pathogenicity and laboratory diagnosis of *S. japonicum* (the oriental blood fluke).**

Morphology

Schistosoma japonicum is morphologically similar to *S. haematobium* except for the following features:

Adult worm

- **Female worm**
 - It is about 2.6 cm × 0.3 mm
 - Its ovary is situated in the middle of the body
 - Its uterus contains 50–300 eggs
- **Male worm**
 - It is about 1.2 cm × 0.5 mm
 - Its body surface is nontubercular—no projections on surface of tegument and therefore body surface is smooth
 - It has seven testes in a single file

Eggs (Fig. 82.1c)

- Shape: Elongated and oval
- Size: Slightly smaller—100 × 65 μ
- It has a lateral small knob

Lifecycle

Needs two hosts to complete the lifecycle.

Definitive host: Human beings

Intermediate host: Fresh water snail of genus *Oncomelania*

Lifecycle is similar to that of *S. haematobium* except for site of localization. They grow into adult worms and become sexually mature in the intrahepatic portion of the portal venous system, then migrate to the superior mesenteric vein down to the capillaries of the last part of ileum, caecum and ascending colon. The eggs are finally excreted in faeces.

Pathogenicity and Clinical Features

- The disease caused is known as **schistosomiasis japonica**; also known as **hepatic and intestinal schistosomiasis of the orient** and **Katayama disease**

- It is similar to and more severe than *S. mansoni* infection because of larger output of eggs:
 - It causes **dysentery** as in *S. mansoni*
 - Central nervous system involvement—causing space occupying lesions—more common
 - Periportal cirrhosis in liver is more severe and common in—*S. japonicum* than *S. mansoni* as it is located near the liver and produces 10 times more eggs

Complications

- Liver fibrosis, intestinal fibrosis, cor pulmonale, portal hypertension are more common in *S. japonicum* than *S. mansoni* infection
- Oesophageal varices, cerebral schistosomiasis

Laboratory Diagnosis

Diagnosis is same as for *S. mansoni* infection; it includes:

- Demonstration of the typical eggs in faeces and in ascending colon biopsy
- Demonstration of antibodies by ELISA
- Demonstration of antigen by ELISA

■ Write in short the treatment for schistosomiasis japonica.

Treatment is same as for *S. haematobium* infection.

■ Describe in brief the morphology, lifecycle, pathogenicity and laboratory diagnosis of *Paragonimus westermani* (oriental lung fluke).

Morphology

Adult worm (Fig. 82.2a)

- It is thick, fleshy, reddish brown (when freshly passed)
- Shape: It is egg shaped
- Size: 7.5–12 mm in length, 4–6 mm in breadth and 4–5 mm in thickness
- It has two suckers—oral near anterior end and ventral near middle of the body
- The worm has life span of 6–7 years

Egg (Fig. 82.2b)

- Shape: Oval
- Size: $80 \times 60 \mu$
- Colour: Golden brown
- Ovum: Unsegmented with a mass of yolk cells

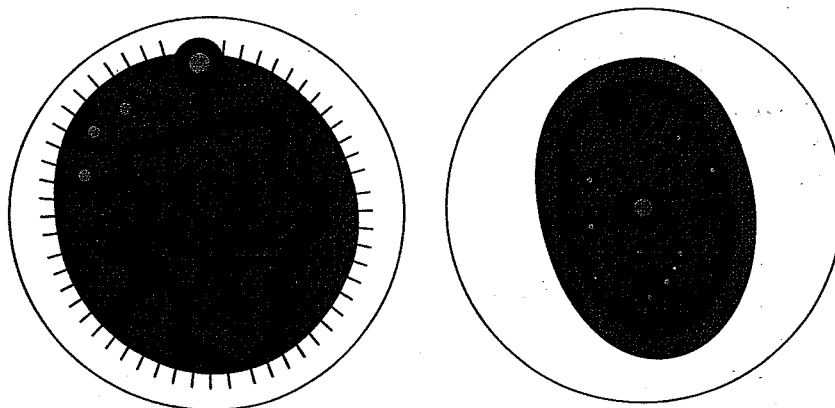


Fig. 82.2 *Paragonimus westermani*: (a) adult worm and (b) egg.

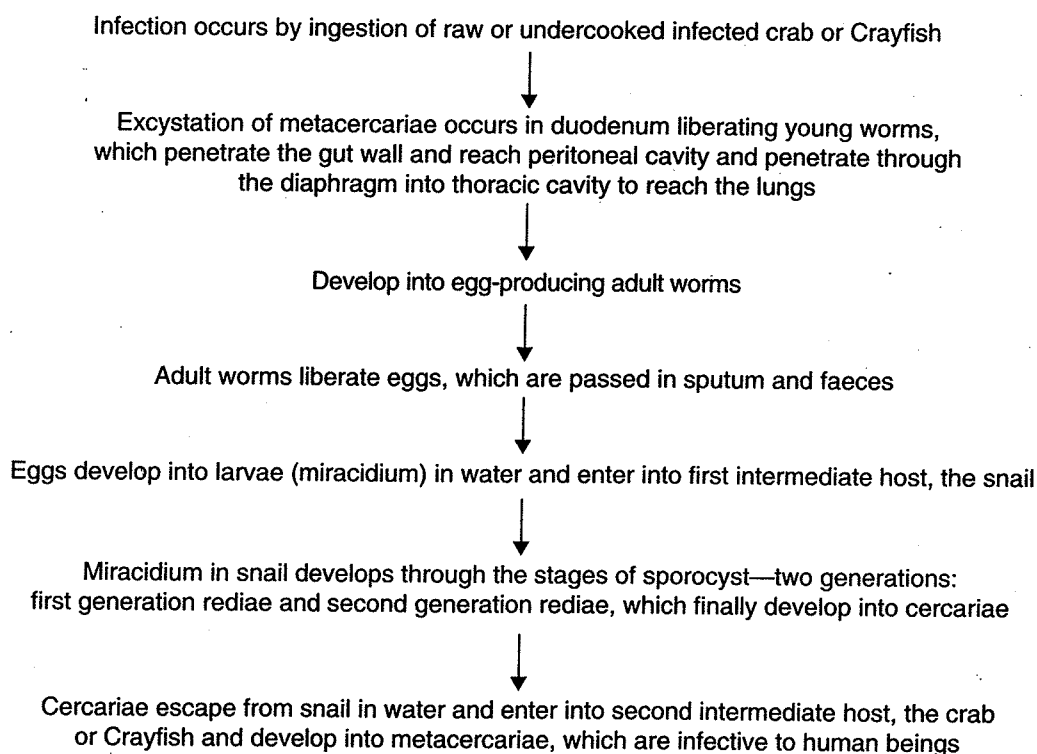
- Eggs are operculated; operculum is clearly visible at larger end, and opposite end without operculum is thickened
- Eggs are excreted in sputum and faeces

Lifecycle (Flowchart 82.2)

Needs three different hosts to complete lifecycle

Definitive host: Human beings

Intermediate hosts: First—Fresh water snails of the genus *Melania*, Second—Fresh water crab or Crayfish



Flowchart 82.2 Lifecycle of *Paragonimus westermani*.

Pathogenicity and Clinical Features

- The disease caused is known as **paragonimiasis**. It occurs as:
 - **Pulmonary paragonimiasis**—characterized by chronic granulomatous reaction because of encystment of eggs in deeper layers of lung and are about one cm in diameter. The lesion shows blood-mixed thick purulent fluid, which consists of golden brown eggs. The cyst may rupture in bronchioles releasing eggs, which are then found in sputum, and if swallowed, in faeces
 Fluke in lungs become encapsulated usually in right lung and cause inflammatory reaction. With progressive destruction of tissue, cavitation occurs around the worm, sputum becomes blood tinged and dark brown in colour
 This clinically manifests as fever, cough, with rusty sputum, dyspnoea, chest pain, haemoptysis and recurrent attacks of bacterial pneumonia
 - **Extrapulmonary paragonimiasis**—may involve abdominal, hepatic, cerebral or subcutaneous tissue. Diarrhoea, abdominal pain, and urticaria due to invasion of intestine and subsequent migration of larvae. Eggs entering in circulation may reach to various organs
 - **Generalized paragonimiasis**: Characterized by fever, generalized lymphadenitis, and cutaneous ulceration

Complications

Complications include lung abscess, pleural effusion, empyema and cerebral paragonimiasis.

Laboratory Diagnosis

It includes:

- Demonstration of eggs in sputum and rarely in stool by saline and iodine preparation
- Demonstration of antibodies by complement fixation test, latex agglutination test, indirect haemagglutination test, counter current immunoelectrophoresis
- Newer tests—ELISA and western blot test are also useful in diagnosis

■ Mention the treatment for paragonimiasis.

- Praziquantel is drug of choice—three times a day for one or two days
- Bithionol is also effective – alternative day for 10–15 days

■ Write a short note on *Fasciola hepatica*.

It is a trematode causing fascioliasis.

- Adult worm - It is a large, flat, leaf shaped and brown coloured fluke, 3 cm × 1.5 cm in size.
- Egg - Operculated, large, ovoid, 130–150 μ × 63–90 μ , bile stained with large, refractile and unsegmented ovum
- Life Cycle - Definitive host: Sheep or other herbivores, human beings are accidental host, Intermediate host: Snails

Infection by ingestion of metacercariae with watercress or aquatic plants. Excystation of metacercariae occurs in duodenum, which then invade liver capsule. Traverse liver parenchyma and settle in common bile duct and develop into adult worm. Adult worms liberate eggs in biliary tract, which reach intestine and passed in faeces. Eggs develop into larvae (miracidium) in water, which enter the snail and develop into sporocyst—two generations: first generation rediae and second generation rediae, which finally develop into cercariae. Cercariae escape from snail in water and encyst on grass, watercress, bark or soil and become metacercariae, which are infective to human beings

- Pathogenicity - The disease caused is known as fascioliasis. It occurs as
 1. Acute infection: traumatic or necrotic lesions leading to hepatomegaly, fever, abdominal pain, nausea, vomiting, diarrhoea and jaundice.
 2. Chronic infection: Adult worm in bile duct causes biliary obstruction, pain, jaundice, secondary bacterial infections.
- Laboratory Diagnosis
 - Demonstration of typical operculated eggs in stool.
 - Demonstration of antibodies—by ELISA, indirect haemagglutination test, indirect immunofluorescence test and western blot test.
 - Molecular techniques such as PCR and DNA probes can also be used.
- Treatment - Bithionol is the drug of choice. Triclabendazole and praziquantel

✓ 51

83

Chapter

Medically Important Intestinal Nematodes

✓ SN.

- Describe the morphology, lifecycle and pathogenicity of *Ascaris lumbricoides*.
Describe the laboratory diagnosis of ascariasis.

Morphology

Adult worm (Fig. 83.1a and b)

- Shape: Cylindrical with tapering ends
- Size: Up to 40 cm in length; it is the largest intestinal nematode
- Anterior end: Thin, shows a mouth surrounded by three finely toothed lips
- Freshly passed worms: Light brown or pink in colour that changes gradually to white
- Body cavity is present, which contains toxic fluid known as ascaron or ascarase or proteose
- It lives for 1–2 years

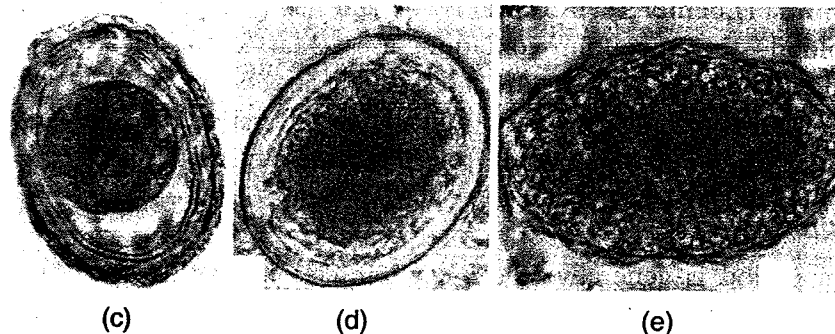
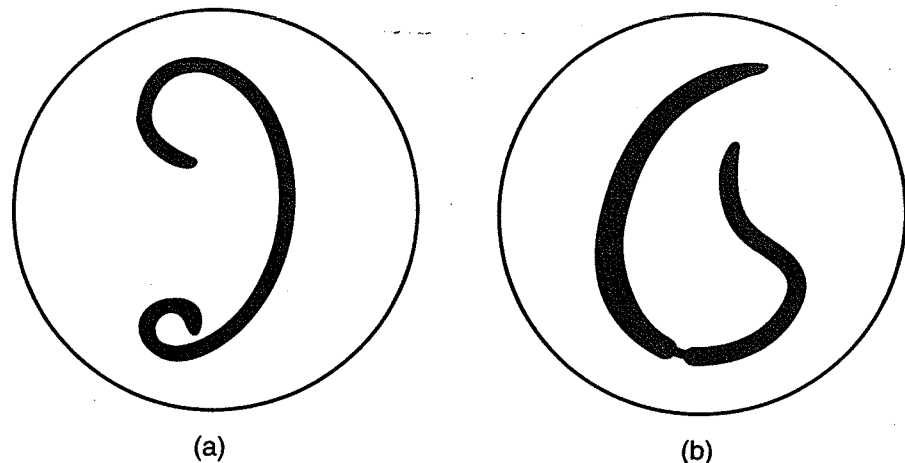


Fig. 83.1 *Ascaris lumbricoides*: (a, b) adult worms, (c) fertilized egg, (d) decorticated egg and (e) unfertilized eggs. (Source: *Journal of Forfar & Arneil's Textbook of Pediatrics*. Fig. 28.59, 2008.)

- **Male worm:** 15–25 cm in length and 2–4 mm in girth with curved posterior (tail) end that has two curved copulatory spicules
- **Female worm:** 25–40 cm in length and 5 mm in girth, stouter than male with straight and conical posterior end. The vulva is situated at the junction of anterior and middle thirds of the body. This part is narrow and is called vulvar waist. It is **oviparous**—**mature female** can liberate about 2,00,000 eggs daily

Eggs

These are of the following two types:

1. **Fertilized egg** (Fig. 83.1c)
 - Shape: Round or oval
 - Size: $60\text{--}70\ \mu \times 40\text{--}50\ \mu$
 - Colour: Yellow-brown—bile stained
 - Egg shell: Thick, smooth, translucent with an uneven outer albuminous coat called as rugosities or mammilliations. This albuminous coat is sometimes absent (decorticated egg, i.e. egg without an outer coat; (Fig. 83.1d)
 - Ovum: Large, unsegmented
 - There is clear, crescentic area between the ovum and the egg-shell at each pole
 - Floats in saturated solution of common salt
2. **Unfertilized egg** (Fig. 83.1e) – Female worm even if not fertilized – liberates eggs
 - Shape: Narrower, longer and more elliptical than fertilized egg
 - Size: $80\text{--}90\ \mu \times 45\text{--}55\ \mu$
 - Colour: Brownish—bile stained
 - Egg-shell: Thinner, with irregular outer albuminous coat
 - Ovum: Small, atrophied ovum with a mass of disorganized, highly refractile granules of various sizes
 - It does not float in saturated solution of common salt

Lifecycle (Flowchart 83.1)

Definitive host: Human beings

Intermediate host: Not required, passes lifecycle in one host, i.e. human beings

✓ Pathogenicity and Clinical Features

The disease caused is known as **ascariasis**. The symptoms are produced by both larvae and adult worms.

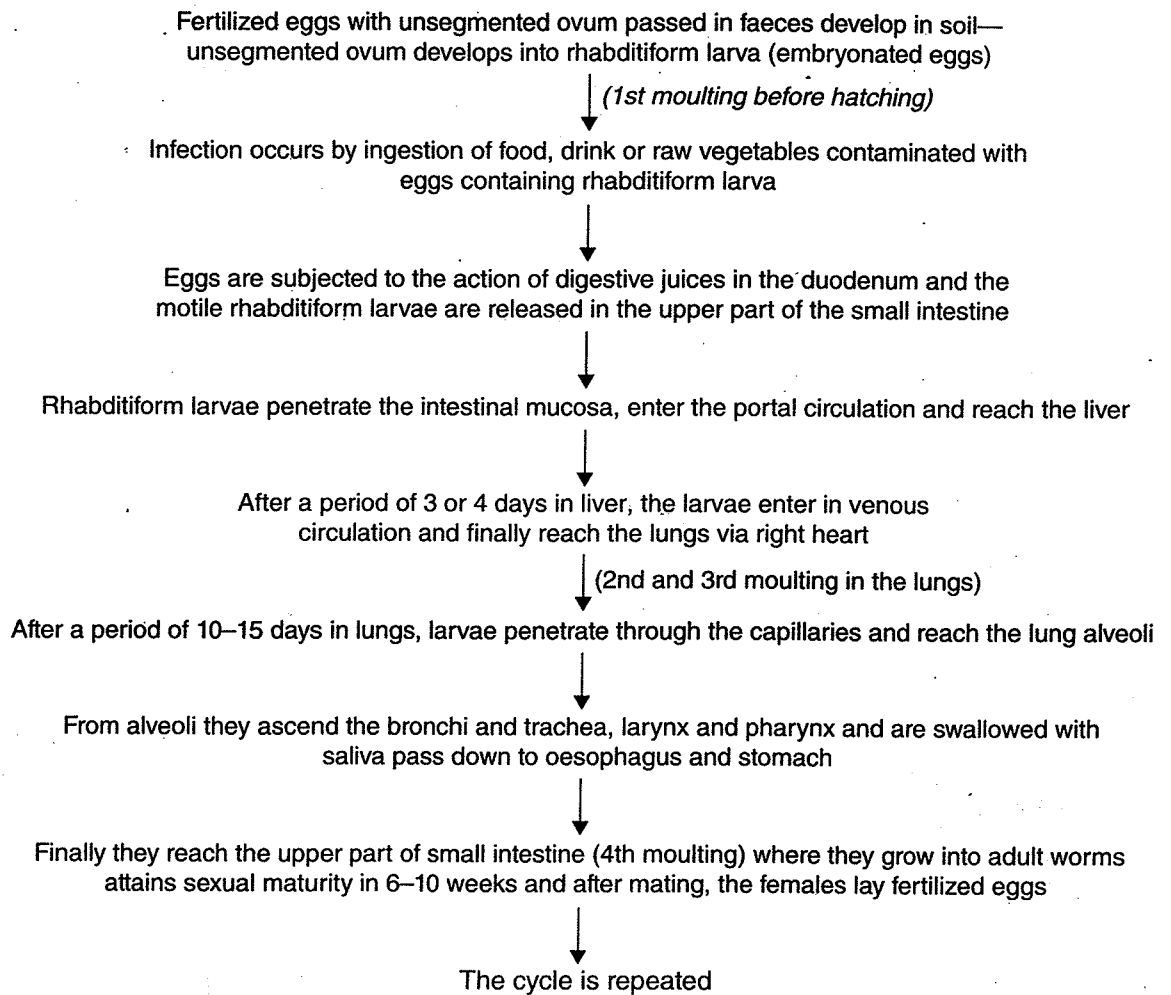
Symptoms due to larvae

Ascaris pneumonia (Loeffler's syndrome) is characterized by fever, dry cough and dyspnoea. Occasionally, blood—tinged sputum-containing ascaris larvae may be expectorated. Urticaria and eosinophilia may also be seen in 20% cases. Rarely larvae may enter the general circulation and reach different organs and may set up unusual manifestation (visceral larva migrans). Migrating larvae may carry microorganisms from intestine to other tissues.

Symptoms due to adult worms

The incubation period is generally 60–75 days. Infection may be asymptomatic. The adult worms produce pathogenic effects in the following ways:

- **Spoliative action:** When present in large numbers, especially in children, they interfere with digestion and absorption of food and cause protein-energy malnutrition and vitamin A deficiency (night blindness)



Flowchart 83.1 Lifecycle of *Ascaris lumbricoides*.

- **Toxic action:** The body fluid of adult worm, if absorbed, produces toxic effects such as typhoid-like fever and hypersensitivity reactions such as fever, urticaria, oedema of face, conjunctivitis and irritation of the upper respiratory tract
- **Mechanical effects:** Presence of large number of entangled worms may cause intestinal obstruction in children, occurrence of intussusceptions and perforation of existing ulcer in the intestine

The adult worm may enter appendix—causing **appendicitis**. It may enter into biliary passage causing **obstructive jaundice** and **acute haemorrhagic pancreatitis**. Rarely, it may penetrate high up in the liver causing **intrahepatic abscess**. It may enter the stomach and pass through the oesophagus and may reach the larynx causing **asphyxia** or may come out through the mouth or nose.

✓ Laboratory Diagnosis

Diagnosis of intestinal ascariasis includes:

- Demonstration of the characteristic eggs in faeces by saline and iodine preparation. Demonstration of eggs in concentrated faeces, if routine examination is negative and demonstration of the adult worms passed in faeces or vomit
- Demonstration of the larvae in sputum or gastric aspirate in ascariasis pneumonia

- Demonstration of worms in intestine or other aberrant sites by plain X-ray, barium emulsion and ultrasound
- Skin test – scratch test – powder from adult worm is used

■ **Write in short the treatment for ascariasis.**

- Piperazine salts (citrate, hydrate, phosphate, adipate)—drug of choice
- Mebendazole and albendazole
- Pyrantel pamoate, hetrazan and thiobendazole can also be used

SN: ■ **Describe the morphology, lifecycle and pathogenicity of Ancylostoma duodenale. Describe laboratory diagnosis of ancylostomiasis.**

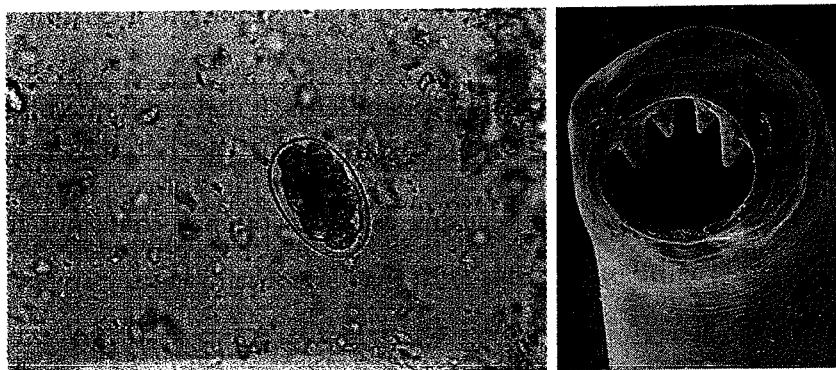
Morphology

Adult worm

- Shape: Small, grayish-white and cylindrical
- Anterior end: Bent dorsally, hence the name hookworm
- Freshly passed worm is reddish-brown because of ingested blood in its intestine
- Mouth has 6 teeth, 4 hook-like on the ventral surface and 2 knob-like on dorsal surface (Fig. 83.2a)
- It lives for 6–8 years
- **Male worm:** Smaller, $8-11 \times 0.4$ mm. Its posterior end has umbrella-like copulatory bursa with two copulatory spicules. The bursa has 3 lobes: 1 dorsal and 2 lateral. These lobes are supported by 13 chitinous rays: dorsal lobe 3 and lateral lobes 5 each
- **Female worm:** $10-13 \times 0.6$ mm. Its posterior end is tapering and no expanded bursa is present like male worm. The vulva opens ventrally at the junction of posterior and middle thirds of the body. It is **oviparous**—mature female can lay 10,000–25,000 eggs daily

Eggs (Fig. 83.2b)

- Shape: Oval or elliptical
- Size: $60 \times 40 \mu$
- Colour: Colourless—nonbile stained
- Egg-shell: Thin transparent hyaline
- Floats in saturated solution of common salt
- Ovum: Segmented ovum generally with 4 or 8 blastomeres – not infective to man



(a)

(b)

Fig. 83.2 *Ancylostoma duodenale*: (a) adult worm—mouth part (Source: *Textbook of Diagnostic Microbiology, Diagnostic Parasitology* Fig. 28-62, 2011.) and (b) egg (Source: *Markell and Voge's Medical Parasitology*, Fig. 8-10, Pages 239-273, Saunders, 2006.)

✓ Lifecycle (Flowchart 83.2)

Definitive host: Human beings

Intermediate host: Not required, passes lifecycle in one host—human beings

Eggs with segmented ova containing 4 or 8 blastomeres, which are passed in faeces develop in soil to form rhabditiform larva and after first and second moulting develops further into filariform larva

Infection occurs by penetration of filariform larva through the skin. Filariform larva on reaching the subcutaneous tissue, enter into the lymphatics or small venules

Larvae pass through the lymph—vascular system into venous circulation and via right heart reach the pulmonary capillaries and enter the alveolar spaces

From alveolar spaces, they migrate to the bronchi, trachea, larynx and pharynx, and are swallowed, on entering into oesophagus (3rd moulting occurs)

Finally they reach the small intestine where they grow into adult worms after 4th moulting, in 3 or 4 weeks, they sexually mature and after mating, the females lay eggs

The cycle is repeated

Flowchart 83.2 Lifecycle of *Ancylostoma duodenale*.

Pathogenicity and Clinical Features

The disease caused is known as **ancylostomiasis (hookworm disease)**. The symptoms are produced by both larvae and adult worms.

Symptoms due to larvae

- **Ground itch or dermatitis**—characterized by pruritic maculopapular dermatitis at the site of entrance of larvae. It is more common with *N. americanus*
- **Creeping eruption**—characterized by a reddish itchy papule along the path traversed by the larval stage of *N. americanus*, *A. brasiliensis* and *A. canium*, which wander aimlessly through the skin layers for several weeks and months producing symptoms termed **larva migrans**. **Bronchitis or bronchopneumonia** may also occur

Symptoms due to adult worms

- During the early phase of the disease, adult worms produce epigastric pain, vomiting and diarrhoea.
- **Anaemia**—microcytic hypochromic anaemia. The causes of anaemia are:
 - Sucking of blood by adult worm through the wound produced in intestinal mucosa during its attachment. A single adult worm of *A. duodenale* sucks 0.2 ml of blood a day and *N. americanus* sucks 0.03 ml blood a day. The sucking of blood is due to the pumping action of oesophagus of worm and is assisted by anti-coagulants in the secretions of the buccal capsule
 - Continuous bleeding from the punctured sites. When the worms change their feeding site, the earlier site continues to bleed for a length of time due to the action of anti-coagulants secreted by worms

- **Nutritional defects**—development of **iron-deficiency anaemia** due to deficiency of the available iron and other haemopoietic substances in the diet, and blood loss due to heavy infection. A hypochromic microcytic anaemia occurs due to deficiency of iron and a microcytic anaemia develops due to deficiency of folic acid and vitamin B₁₂. Dimorphic anaemia occurs when there is deficiency of both iron and vitamin B₁₂ or folic acid. Malnourished individuals may have **hypoproteinaemia**

Laboratory Diagnosis

Diagnosis includes:

- Demonstration of the characteristic eggs in faeces by saline and iodine preparation and in concentrated faeces, if routine examination is negative. Demonstration of the adult worms passed in faeces spontaneously or after a vermifuge and in duodenal contents obtained by duodenal intubation may also be possible sometimes
- Egg counting to find out the intensity of infection can be used—more than 50 eggs per milligram of faeces indicate massive infection. Stool examination for occult blood and Charcot–Leyden crystals

■ Write in short the treatment for ancylostomiasis.

- Mebendazole is effective against both larvae and adult worms – it is given twice a day for three days
- Albendazole (single dose), pyrantel pamoate (for three days), thiabendazole and levamisole are also effective
- Oral iron and appropriate nutritional support for the correction of hypoproteinaemia and anaemia

■ Write a short note on *N. americanus*.

N. americanus is similar to *A. duodenale* in many aspects with slight differences in morphological features. These are as follows:

- Shape: Smaller and more slender than *A. duodenale*
- Anterior end: Bends in opposite direction
- Buccal capsule has 4 chitinous plates—2 on ventral surface and 2 on dorsal surface
- **Male worm:** 7–9 × 0.3 mm. Copulatory bursa is long and wide with 2 copulatory spicules fused at the tip. The bursa has 14 rays of which the dorsal ray is split from the base—dorsal lobe—3 rays and lateral lobes 5 rays each
- **Female worm:** 9–11 × 0.4 mm. The vulva is placed in the middle of the body
- Its lifecycle, morphology, pathogenicity, diagnosis, treatment and prophylactic measures are similar to *A. duodenale*, but *N. americanus* is comparatively less pathogenic than *A. duodenale* (Fig. 83.3a and b)

SN ■ Describe the morphology, lifecycle, pathogenicity and laboratory diagnosis of *Trichuris trichiura* (whipworm).

Morphology

Adult worm (Fig. 83.4a)

- Shape: Pinkish-white, resembles a whip. The anterior three-fifth is thin, hairlike and coiled. The posterior two-fifth is thick and stout—resembles the handle of whip—**whip worm**
- Anterior portion contains a long capillary oesophagus and posterior part contains the intestine and reproductive organs
- It lives in intestine for many years

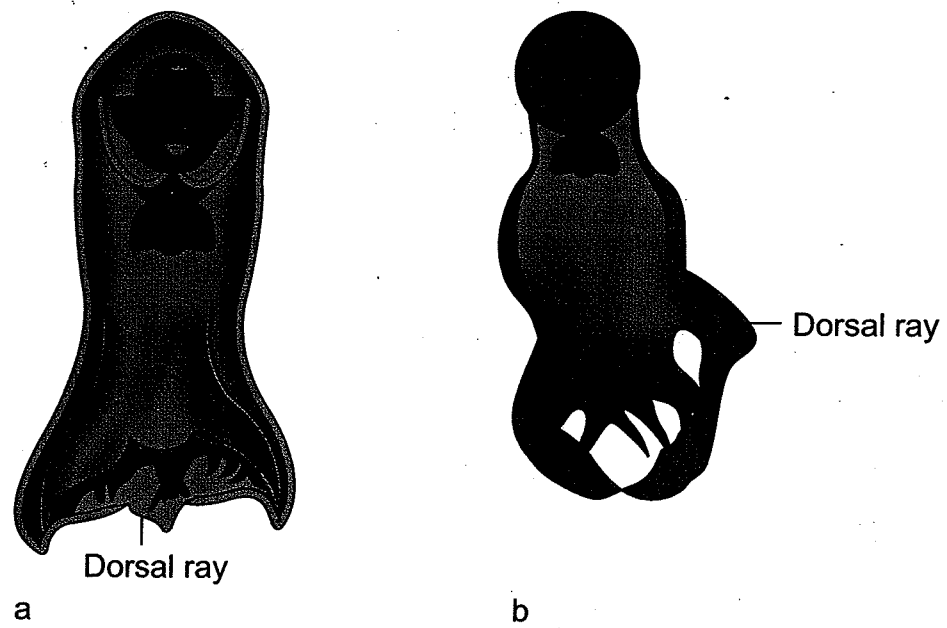


Fig. 83.3 Differentiating features of (a) *Ancylostoma duodenale* and (b) *Necator americanus*.

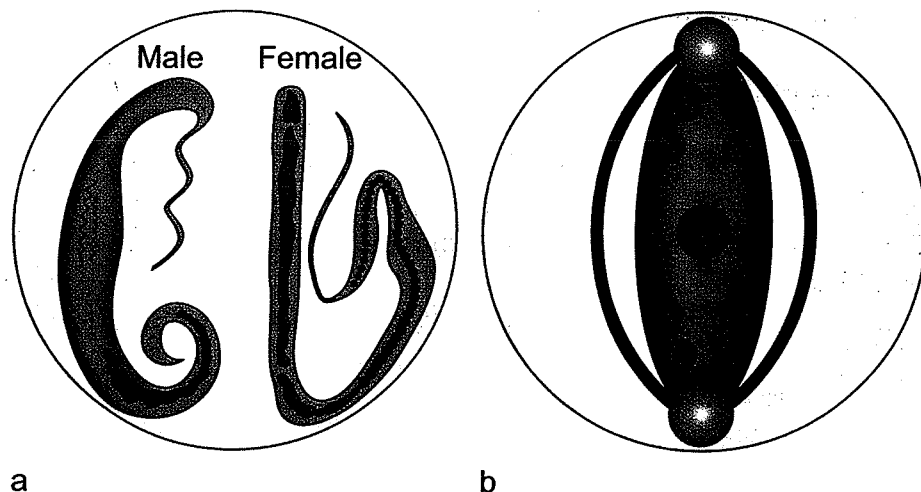
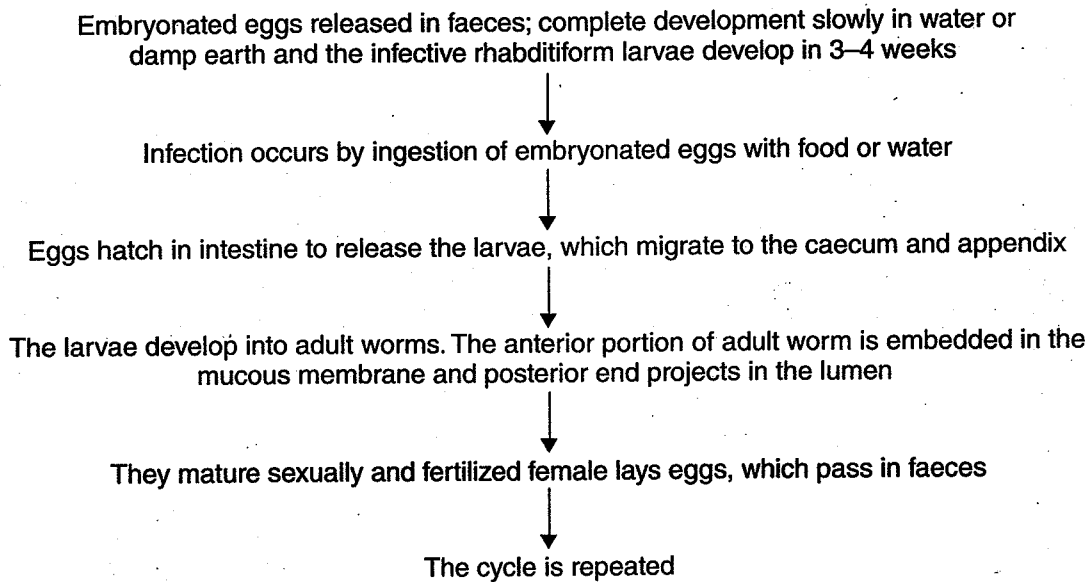


Fig. 83.4 *Trichuris trichiura*: (a) adult worms and (b) egg.

- **Male worm:** 3–4 cm long. The posterior end is coiled and has a single spicule
- **Female worm:** 4–5 cm long. The posterior end is blunt and rounded. It is **oviparous**—mature female lays about 5,000 eggs every day

Eggs (Fig. 83.4b)

- **Shape:** Barrel shaped with projecting mucous plugs at both poles
- **Size:** $50 \times 25 \mu$
- **Colour:** Brown—bile stained and contains double shell
- **Ovum:** Unsegmented ovum
- It floats in saturated solution of common salt

Lifecycle (Flowchart 83.3)**Definitive host:** Human beings**Intermediate host:** Not required, passes lifecycle in one host—human beings**Flowchart 83.3** Lifecycle of *Trichuris trichiura*.**Pathogenicity and Clinical Features**The disease caused is known as **trichuriasis**

- Usually, the worms do not produce any pathogenic effects and asymptomatic
- Heavy infections are characterized by the abdominal pain, mucoid diarrhoea often with blood streaked stool and weight loss
- It may lead to anaemia, malnutrition and growth retardation
- Rarely prolapse of rectum in massive infection in children
- Symptoms of acute appendicitis

Laboratory Diagnosis

Diagnosis includes:

- Adult worms in faeces may be seen occasionally
- Demonstration of the characteristic eggs in faeces

Write in short the treatment for trichuriasis.

Mebendazole (twice a day for three days) or albendazole (single dose).

Describe the morphology, lifecycle, pathogenicity and laboratory diagnosis of *Enterobius vermicularis* (pinworm, threadworm, seatworm).**Morphology****Adult worm (Fig. 83.5a)**

- Shape: Small, white, spindle-shaped; resembles a piece of white thread
- A pair of wing-like expansions at the anterior end called **cervical alae** are present
- A double bulb oesophagus is the characteristic feature
- No buccal cavity is present

- **Male worm:** $2-4 \times 0.1-0.2$ mm. The posterior end is curved and has a coiled tail with a single spicule. It dies after fertilization of the female
- **Female worm:** $8-13 \times 0.3-0.5$ mm. The posterior third of the body is long tapering pointed tail. It is **oviparous**—mature female lays eggs and after oviposition dies in 2 or 3 weeks

Eggs (Fig. 83.5b)

- Shape: Planoconvex—flattened on ventral side and convex on dorsal side
- Size: $50-60 \mu \times 30 \mu$
- Colour: colourless—non-bile stained
- Egg-shell: Transparent
- Egg contains - a coiled tadpole-like larva
- It floats in saturated solution of common salt

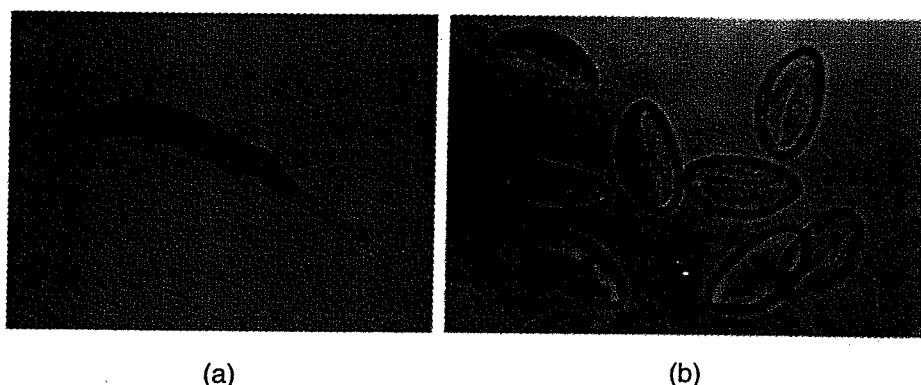
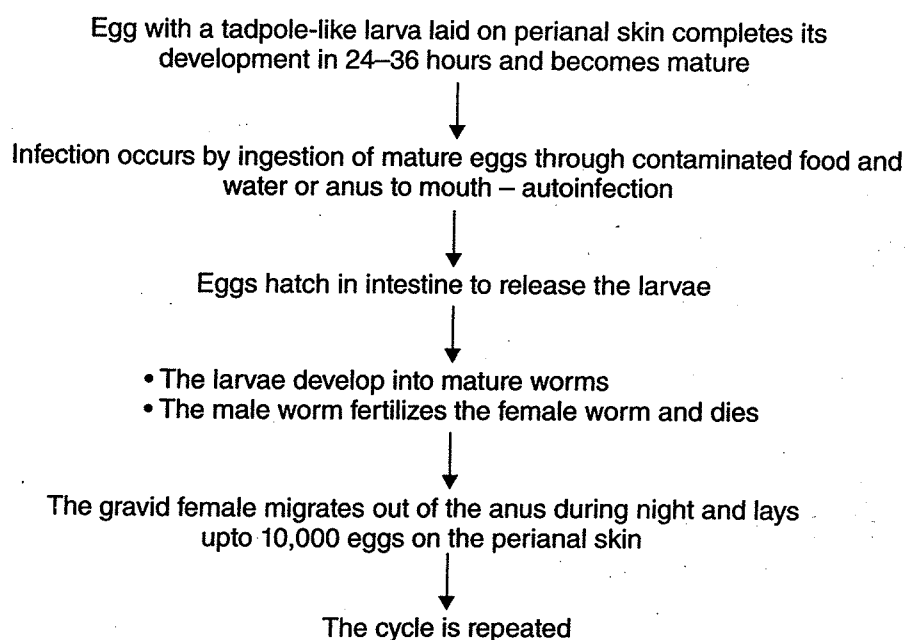


Fig. 83.5 *E. vermicularis*: (a) adult worm and (b) egg. (Source: Tropical Infectious Diseases: Principles, Pathogens and Practice, Pages 788-790, Saunders, 2011.)

Lifecycle (Flowchart 83.4)

Definitive host: Human beings

Intermediate host: Not required, passes lifecycle in one host—human beings



Flowchart 83.4 Lifecycle of *Enterobius vermicularis*.

Pathogenicity

The disease caused is known as enterobiasis.

- It is common in children. Familial infection is common
- Infection occurs by the ingestion of eggs
- The eggs deposited on perianal skin contaminate nightclothes and bed linens of infected persons
- Infection can be transmitted due to contamination of hands by handling such nightclothes and bed linens
- Infection can also occur by inhalation of eggs that become air borne during bed making
- Reinfection of same host (autoinfection) is also possible, it occurs in the following two ways:

1. By Hand-to-Mouth

In which fingers get contaminated with eggs because of scratching of affected itching part around the anus. These eggs are then either transferred to food articles and swallowed or transferred directly from anus to mouth.

2. Retrograde Infection

In which eggs laid on perianal skin hatch out into larvae, which migrate back through the anus up to colon and develop into adult worms.

Clinical Features

- Perianal pruritus and an eczematous condition round the anus and perineum
- Nocturnal enuresis
- Inflammation of the vermiform appendix may also occur
- Invasion of female genital tract may occur rarely, causing vulvovaginitis, salpingitis and pelvic or peritoneal granulomas

Laboratory Diagnosis

Diagnosis includes:

- Demonstration of the adult worms in faeces after a purge or an enema
- Demonstration of characteristic eggs in faeces by direct smear examination or in concentrated faeces, where it may be negative as eggs are deposited on the perianal skin. Hence, demonstration of eggs in the scrapings from the perianal skin by NIH swab or cellophane tape is a better method. NIH (National Institute of Health) swab, a cellophane anal swab, can be used for collection of specimen. It consists of 8–10 cm long by 4 mm wide glass rod covered with piece of transparent cellophane of about one inch square held in place by a rubber band used for swabbing. The other end of the rod passes through the rubber cork used to close the test tube (Fig. 83.6). After taking the swab, the cellophane with the rod is placed in tube and sent to laboratory. For examination, a drop of saline is taken on a glass slide; cellophane is released on slide by pushing rubber band with the help of forceps. The cellophane is spread out and smoothened on a glass slide. A drop of saline is placed over it and observed under low power and high power objectives after placing a cover slip

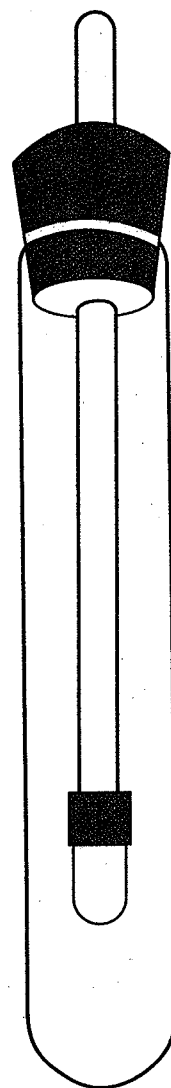


Fig. 83.6 NIH swab.

- Demonstration of eggs in washings of finger tips, linen and garments
- The worms may be discovered by the patient or parents of the children
- Inspection of perianal region at the commencement of itching may reveal gravid female

■ **Write in short the treatment for enterobiasis.**

Mebendazole or pyrantel pamoate.

84

Chapter

Medically Important Tissue Nematodes

■ Enumerate the medically important tissue nematodes.

Following are the medically important tissue nematodes:

- *Wuchereria bancrofti* causing lymphatic filariasis
- *Brugia malayi* causing lymphatic filariasis
- *Loa loa* causing loiasis
- *Onchocerca volvulus* causing onchocerciasis
- *Dracunculus medinensis* causing dracunculosis

37 ■ Discuss in brief the morphology, lifecycle, pathogenicity and laboratory diagnosis of *Wuchereria bancrofti* (Bancroft's filaria).

Morphology

Adult worm

- Long, thread-like, transparent, creamy white in colour and filariform in shape with smooth surface
- Their ends are tapering and rounded. Anterior end is slightly swollen
- Its average life span is 4–5 years
- **Male worm:** 2.5–4 cm in length and 0.01 mm in thickness. The tail end is curved ventrally and contains two spicules of unequal length
- **Female worm:** 8–10 cm long and 0.2–0.3 mm in thickness. Tail end is narrow and abruptly curved. It is **ovo-viviparous** and liberates eggs with well-developed sheathed embryos (microfilariae)

Embryo (*Microfilaria*; Fig. 84.1)

- It is a first stage larva, found in peripheral blood, hydrocele fluid and chylous urine
- **Size:** 275–300 μ long and 8–10 μ in breadth
- **Motility:** Actively motile with the help of coiling and uncoiling of its tail
- **Hyaline sheath:** It is a structureless sac covering the embryo, it is longer than the larval body and measures 350 μ . The larva moves forward and backward in sheath



Fig. 84.1 *Wuchereria bancrofti*: microfilaria. (Source: *Journal of Topics in Companion Animal Medicine*. Fig. 1, Pages 160-172, 2011.)

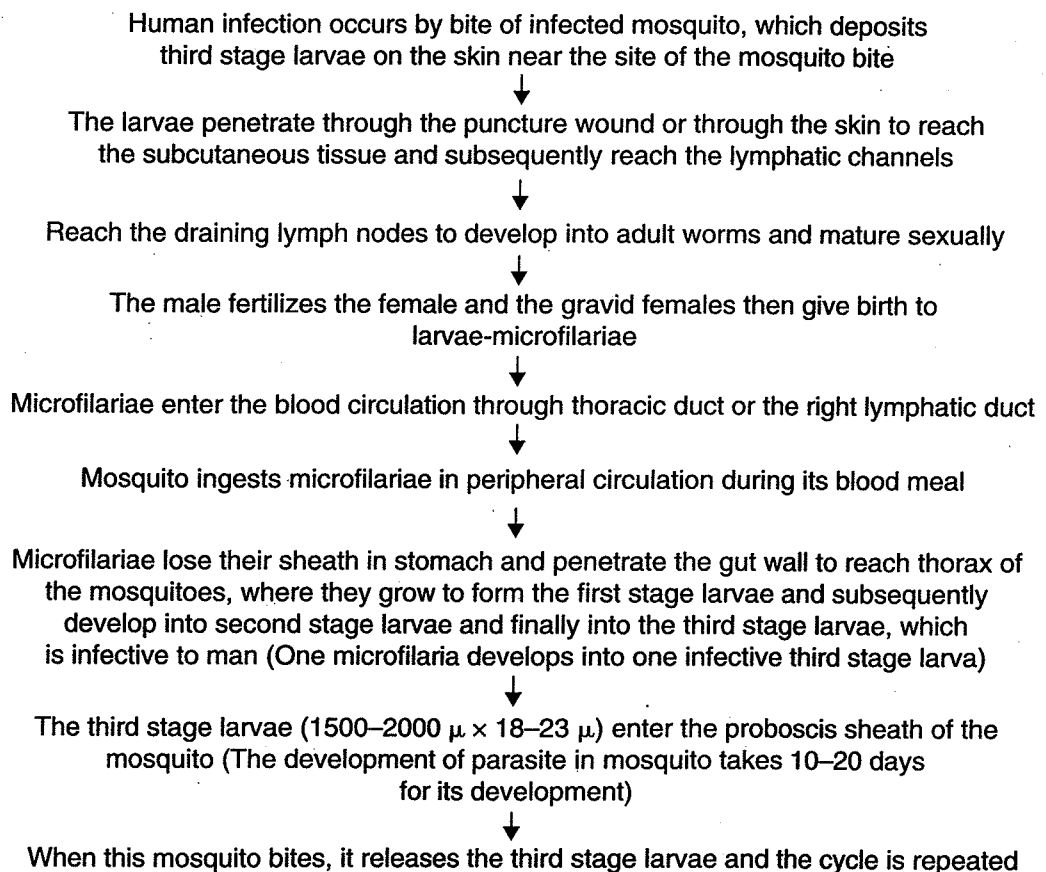
- Head end is blunt and tail end is tapering
- In unstained preparation, it appears transparent and colourless
- In a stained smear (Romanowsky's stain), it is stained pink with a column of violet nuclei (somatic cells) extending from head to tail end but absent at the tip of the head and tail. At the anterior end (head end), there is a space devoid of granules (nuclei), it is known as the **cephalic space**. The column of nuclei is broken at definite places by organs such as nerve ring, excretory pore, excretory cell, genital cells and anal pore. Location of these organs is different in different species, hence it serves as important mark for identification
- Genital cells are G1, 2, 3 and 4; G2, 3, 4 are present just in front of anal pore and G1 is situated farther in the front
- Innenkorper is extended from excretory pore to G1
- Microfilariae do not undergo further development in human body unless they are taken by mosquito

Lifecycle (Flowchart 84.1)

It is completed in two different hosts

Definitive host: Human beings

Intermediate host: Mosquitoes of genus *Culex*, *Aedes* and *Anopheles*



Flowchart 84.1 Lifecycle of *Wuchereria bancrofti*.

Pathogenicity and Clinical Features

The disease caused -wuchereriosis (Bancroftian or lymphatic filariasis)

- The pathogenic and clinical effects are due to inflammatory damage to the lymphatics by adult worms
- Clinically it occurs as lymphatic filariasis and occult filariasis

Lymphatic Filariasis or Classical Filariasis

Stages responsible for pathogenesis are as follows:

1. **Dilatation of lymph vessels**—Inflammatory reaction provoked by adult worm, developing larvae, metabolic products released during moulting and immune reaction of host are responsible for dilatation of lymphatic vessels
2. **Infection of lymph vessels (Lymphangitis)**—It is characterized by the presence of dilated, inflamed, thick lymph vessels associated with oedema, erythema and tenderness. Dilatation of lymphatics also leads to lymphoedema and thickening of endothelium
3. **Obstruction to lymph nodes**—Fibrotic degenerative changes in lymphatic vessels proximal to lymph node occurs. Lymph node also shows sclerosis and obstruction to lymphatic channels

Clinical Manifestation of Filariasis

- **Acute filariasis:** It is characterized by filarial fever, lymphoedema due to presence of adult worm in lymphatic channels, thereby interfering with flow, lymphadenitis (characterized by enlarged, tender lymph nodes) and adenolymphangitis (inflammation of lymphatic channels). Fever is usually of low grade and associated with chills and generalized malaise and headache
- **Chronic filariasis:** It develops after 10 years of infection, during this inflammation subsides and fibrosis advances. It clinically manifests as:
 - **Lymph varices**—These are varicose lymph ducts, formed due to dilatation of lymph vessels because of obstruction. Lymphangiovarices rupture may lead to chyluria, chylothorax, chylous ascites and chylous diarrhoea. Obstruction to lymph vessels of spermatic cord leads to hydrocele
 - **Elephantiasis**—It occurs due to complex immune reaction and repeated superinfections. In males, the arms, legs and scrotum are affected. In females, the arms, legs, vulva and breast are affected. The affected part becomes enormously enlarged and overlying skin becomes thickened, fissured and even papillomatous. Secondary bacterial infections may occur

Occult Filariasis

It is a condition of hypersensitivity to filarial antigen. Microfilariae are not found in peripheral blood and other features of filariasis are absent. Tropical pulmonary eosinophilia is the most common manifestation and is characterized by hypereosinophilia of peripheral blood and increase in filarial antibodies along with IgE (Table 84.1).

Table 84.1 Differences between classical and occult filariasis

	Classical filariasis	Occult filariasis
Causative agent	Adult worm	Microfilariae
Pathology	Lymph vessels and lymph nodes are affected	Lymph vessels, lungs and liver are affected
Clinical features	Lymphangitis and lymphadenitis	Eosinophilic granuloma and hypersensitivity
Microfilariae	Present in blood	Absent in blood
Filarial antibodies	Demonstration—not diagnostic	Demonstration of increasing titre is diagnostic

Laboratory Diagnosis

Diagnosis includes:

- Demonstration of the microfilariae in peripheral blood
 - **Time of blood collection:** Blood should be collected between 10 p.m. to 4 a.m., as *W. bancrofti* has nocturnal periodicity, alternatively blood can be collected during day-time to avoid inconvenience to the patient by giving diethylcarbamazine (100 mg) orally and collecting and examining blood for microfilariae in 30–45 minutes (diethylcarbamazine provocation test)
 - **Site for collection:** Blood should be collected from ear lobes as number of microfilariae is more as compared to fingers (also they are more in number in capillary blood than in venous blood)
 - **Methods for demonstration:** Microfilariae in blood can be demonstrated in:
 - A wet mount (unstained preparation)—motile microfilariae can be seen under low power
 - Stained preparation—in a thin or thick blood film stained with Leishman's stain or Romanowsky's stain
- Demonstration of microfilariae in the chylous urine, exudates of lymph varix, hydrocele fluid
- Demonstration of the adult worm in lymph node biopsy and calcified worm by X-ray
- Demonstration of Abs using nonspecific Ags can be used. The various tests used for serological diagnosis are passive haemagglutination test, fluorescent antibody test, complement fixation test, ELISA and RIA. These tests show cross reactivity with other filarial and helminthic infections and cannot differentiate past and current infection
- Demonstration of circulating Ag in serum can be detected by using ELISA. The Ag is present during recent or current infection only
- Molecular methods such as polymerase chain reaction can also be used for diagnosis
- Skin test is positive in infected cases
- Xenodiagnosis – demonstration of microfilariae in the stomach blood of mosquito vector, which was allowed to bite infected individual

■ Write in short the treatment for wuchereriosis.

Diethylcarbamazine is effective against microfilariae; long-term (three weeks) low dose therapy is required to damage adult worm and cure the disease completely.

■ Discuss in the brief morphology, lifecycle, pathogenicity and laboratory diagnosis of *Brugia malayi* (*Wuchereria malayi*, Malayan filaria).

Morphology

Morphologically *Brugia malayi* is similar to *W. bancrofti*. The differentiating features are given in Table 84.2.

Lifecycle

It completes lifecycle in two different hosts

- **Definitive host:** Human beings
- **Intermediate host:** Mosquitoes of genus *Mansonia* spp.
- Lifecycle is similar to that of *W. bancrofti* except for the intermediate host

Pathogenicity and Clinical Features

The disease caused is known as Malayan filariasis. Clinically it is similar to *W. bancrofti* infection with some variations, which are as follows:

- As compared to *W. bancrofti* lymphangitis (inflammation of lymphatic channels), and filarial abscesses occur with a greater degree of frequency

Table 84.2 Differentiating features of *B. malayi* and *W. bancrofti*

Features	<i>W. bancrofti</i>	<i>B. malayi</i>
Adult worm		
1. Size	Bigger	Smaller
2. Principal vector	<i>Culex</i> mosquito	<i>Mansonia</i>
3. Elephantiasis	More common	Less common
Microfilariae		
1. Length	250–300 μ	175–230 μ
2. Diameter	8 μ	6 μ
3. Sheath	Present, stains lightly with Giemsa stain	Present, stains deeply with Giemsa stain
4. Body curve	Regular, sweeping curves	Kinky, irregular curves
5. Cephalic space	Length and breadth equal	Almost twice as long as broad
6. Stylet at anterior end	Single	Double
7. Excretory pore	Not prominent	Prominent
8. Nuclear chromatin	Discrete nuclei	Blurred
9. Tail tip	Pointed, free of nuclei	Tapering with two nuclei, one at the tip and another subterminal

- In contrast to *W. bancrofti*, elephantiasis of the genitalia is seldom seen and if at all it occurs, it is restricted to the lower extremities below the knee
- Sclerotic cord-like lymphatics and lymphadenopathy in arms and legs are common

Laboratory Diagnosis

It is same as for *W. bancrofti*.

■ Write in short the treatment for Malayan filaria.

- Similar to *W. bancrofti*
- Diethylcarbamazine—is the drug of choice

■ Describe the morphology, lifecycle, pathogenicity and laboratory diagnosis of *Dracunculus medinensis*.

Morphology

Adult worm (Fig. 84.2a)

- **Male worm:** 2–4 cm in length and 0.4 mm in breadth
- **Female worm:** 70–120 cm long with a diameter of 1.5–1.7 mm. It resembles a piece of long twine thread and is milky white in colour. It is **viviparous**

Larvae (Embryos) (Fig. 84.2b)

- Size: 500–700 μ long and 17–20 μ in breadth
- They are coiled bodies with rounded heads and long slender tapering tails
- They are actively motile with the help of coiling and uncoiling of its tail

Lifecycle (Flowchart 84.2)

It completes lifecycle in two different hosts

Definitive host: Human beings

Intermediate host: Cyclops (Fig. 84.2c)—*Mesocyclops leuckarti*

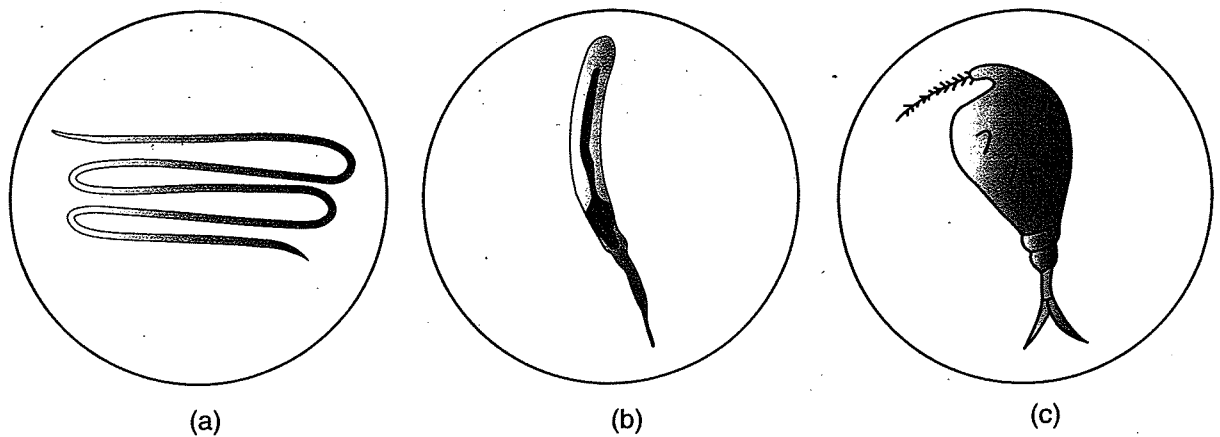
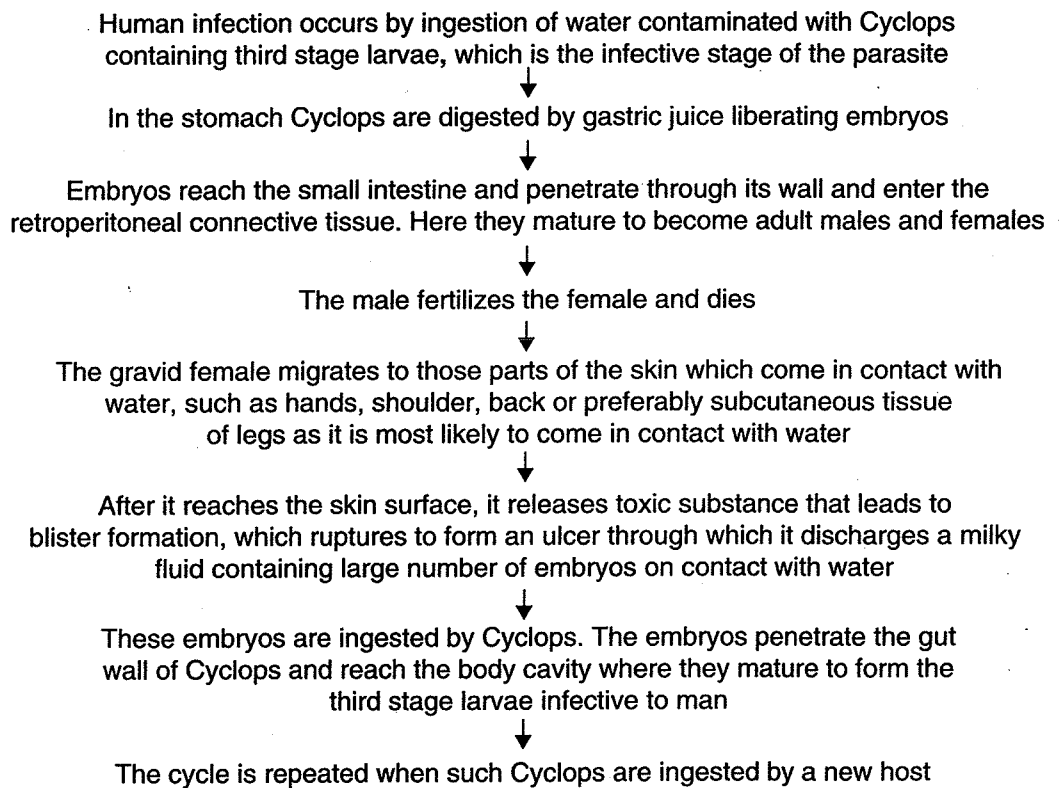


Fig. 84.2 *Dracunculus medinensis*: (a) adult worm, (b) embryo and (c) cyclops.



Flowchart 84.2 Lifecycle of *Dracunculus medinensis*.

Pathogenicity and Clinical Features

The disease caused is known as dracunculosis (guinea worm disease)

- The symptoms are produced during parturition of the female worm
- The symptoms include: Allergic manifestations and blister formation due to the liberation of toxic substances
- The worm tries to come out through the skin to discharge embryos. This leads to itching and burning sensation, pain, swelling and a small bleb or **blister formation** on the skin
- This generally occurs in lower extremities but may sometimes occurs at other sites such as hands, shoulder, back, trunk, buttock, scrotum, neck and female breast

- Finally, the blister ruptures, leaving behind a shallow ulcer with a tiny round hole in the centre through which the worm releases embryos, whenever this part comes in contact with water. The female continues to discharge embryos till the stock is exhausted
- Sometimes, the worm may not reach the skin and dies in deeper tissue, which encapsulate and later calcify
- Sometimes there may be formation of abscess in tissue and painful arthritis may occur

Laboratory Diagnosis

Diagnosis includes:

- Demonstration of the adult worm at the surface of the skin
- Demonstration of the embryos in milky fluid released by the worm on exposure to water
- Demonstration of the calcified worm accidentally on X-ray
- In atypical cases, serological diagnosis is done by ELISA test
- Skin test

■ Write in short the treatment for dracunculosis.

- No specific and effective drug is available
- Thiabendazole or metronidazole gives symptomatic relief
- The extraction of the worm is carried out by winding the worm to a matchstick and pulling it inch-by-inch daily by applying or bathing affected part with cold water until the whole parasite comes out. This gradual extraction takes about 15–20 days. Alternatively, it can be excised surgically

UNIT

VII

Clinical Microbiology

85

Chapter

Septicaemia and Bacteraemia

■ Explain septicaemia.

Septicaemia literally means 'sepsis of blood'. It is a condition in which there is presence of actively multiplying bacteria in the blood stream, and formation of toxic products in the blood.

■ Define pyaemia.

Pyaemia is septicaemia caused by pyogenic bacteria with multiple abscesses in internal organs such as spleen, lungs, liver, kidneys, brain, heart, etc.

■ What is toxæmia?

Toxæmia refers to formation of toxic products in blood. When bacterial endotoxins circulate in the blood, the condition is called endotoxæmia. Endotoxæmia can occur in absence of bacteria in the blood stream, e.g. from Gram-negative bacteria present in the gut, endotoxin passes across the gut wall.

■ Define bacteraemia.

Bacteraemia is a condition in which there is transient presence of bacteria in blood stream without multiplication. Clinical signs of septicaemia are absent.

■ Enumerate the common microorganisms that cause septicaemia and bacteraemia.

Following are the microorganisms causing septicaemia and bacteraemia:

Bacteria

Common Bacterial Agents

- *Escherichia coli*
- *Streptococcus pneumoniae*
- *Staphylococcus aureus*
- *Klebsiella* spp.
- *Proteus* spp.
- *Haemophilus influenzae*
- *Salmonella* spp.
- *Streptococcus pyogenes*
- *Pseudomonas aeruginosa*
- Group B Streptococci
- *Str. viridans*
- *Staph. epidermidis*

Other Bacterial Agents

- Enterococci
- *Enterobacter aerogenes*
- *Citrobacter* spp.
- *Acinetobacter* spp.
- *Bacteroides* spp.
- Anaerobic cocci—peptococci and peptostreptococci
- *Neisseria meningitidis*
- *Brucella* spp.
- *Rickettsia* spp.
- *Coxiella* spp.

Fungi

- *Candida albicans* and other species
- *Cryptococcus neoformans*
- *Mucor* spp.
- *Aspergillus* spp.
- *Histoplasma capsulatum*
- *Coccidioides immitis*
- *Blastomyces dermatitidis*

■ Briefly discuss the pathogenesis of septicaemia.

- The vascular compartment of the body is sterile. Microbes may enter the bloodstream from an infective focus with the help of phagocytic cell or from a surface with normal flora causing breakage of blood vessels or by introduction of contaminated material directly into the vascular system
- Organisms that enter the bloodstream are quickly eliminated by various immune mechanisms. But when the immune system is overwhelmed or evaded, the microorganisms persist in the blood and multiply producing signs and symptoms of septicaemia

■ Mention the clinical features and complications of septicaemia.

Characteristics of septicaemia are as follows:

Clinical Features

- Fever or hypothermia
- Rigors
- Tachycardia
- Tachypnoea
- Hypoxia
- Dyspnoea
- Cyanosis
- Hypotension
- Mental confusion
- Agitation in elderly patients
- Behavioural changes

Complications

- Septic, endotoxic or bacteraemic shock
- Disseminated intravascular coagulation (DIC)

- Acute renal failure
- Shock may lead to multiple organ failure (e.g. heart, lungs, liver, kidneys)

■ Describe the laboratory diagnosis of septicaemia/bacteraemia.

Blood Culture

Collection of Blood

- Apply 2% tincture iodine to the skin over the area of vein and allow it to dry
- Remove the iodine with the help of sponges saturated with 80% isopropyl alcohol
- The entire procedure is repeated and tourniquet is applied
- Collect 10 ml of blood with the help of sterile syringe and needle
- The blood has to be diluted (1:10) to inactivate antimicrobial components present in the blood. Hence, 5 ml of blood is collected in 50 ml of glucose broth and 5 ml of blood is collected in 50 ml of bile broth. (Total 10 ml of blood is collected as the number of organisms in the blood may be very less, may be 1/ml)
- Brain–heart infusion broth, trypticase soya broth and even nutrient broth can be used for collection
- For anaerobes—thioglycollate broth or Robertson's cooked meat medium can also be used
- Sodium polyanethol sulphonate (SPS) is added to broth media, which acts as an anticoagulant, antiphagocytic and anticomplementary. It interferes with the activity of certain antimicrobial agents such as aminoglycosides, enhances growth rate and increases the rate of isolation

Processing of Blood Culture

- After collection of blood, blood culture bottles are incubated aerobically and blind subcultures are made after 24 hours, 48 hours, 72 hours, on 6th day and 10th day
- The subcultures are made on
 - Blood agar
 - MacConkey's agar
 - Chocolate agar
 - Anaerobic supplemented blood agar for anaerobes
 - Sabouraud's dextrose agar for fungi
 - Other selective media as indicated
- One blood agar is incubated anaerobically, chocolate agar under 5–10% CO₂ and other media under ordinary conditions. After incubation, colonies obtained on solid media are studied further for identification by using biochemical reactions and antibiotic susceptibility by Kirby–Bauer's disc diffusion method

Castaneda's Method

- Alternatively, Castaneda's method is used for rapid detection of causative agents.
- The blood specimen is collected into liquid medium and bottle is incubated in upright position. For inoculation of agar, bottle is tilted at intervals so that the broth flows over the agar slant. Again it is incubated in upright position. The colonies formed on agar slant are studied and identified (See Fig. 44.1)

Automated Blood Culture System

- Various newer unconventional methods for detecting bacteria in blood are now available which detect bacterial growth by various techniques such as the detection of carbon dioxide by radiometric or optical methods. One such instrument commonly used is *BACTEC System*.

Cultures of Other Specimens

Cultures of following specimens may be useful in the diagnosis of septicaemia:

- Culture of intravenous catheter tips
- Swab from infected burns
- Swab from wounds or abscesses
- Urine in UTI
- Sputum in respiratory tract infections
- Stool in intestinal infections

Nonculture Methods

Various nonculture methods are used to detect circulating antigens and other microbial products. These are, as follows:

- **Latex agglutination test**—used for detecting antigen of Group B Streptococci, *H. influenzae* Type b, *Str. pneumoniae*, *N. meningitidis*, staphylococci and some yeasts
- **Counter current immunoelectrophoresis (CIEP)**—used in detection of pneumococci, *Klebsiella*, *H. influenzae* and other microbial agents
- **Limulus amoebocyte lysate assay test**—used to detect circulating lipopolysaccharide (endotoxin) of Gram-negative bacteria in blood

■ Mention the ways in which septicaemia/bacteraemia can be treated.

Septicaemia/bacteraemia can be treated in the following ways:

- Immediate parenteral antibiotic therapy (IV route is the best) in adequate dosage—necessary as soon as specimen for culture is collected
- Surgical intervention—urgent surgical drainage of abscesses, wounds, etc.
- Intravenous fluids to prevent shock
- Blind drug therapy is given before the availability of antibiotic susceptibility testing report, based on the
 - Clinical symptoms and signs
 - Likely focus and cause of infection
 - Knowledge of the underlying conditions
- The correct antibiotic therapy is started after the availability of antibiotic susceptibility testing report
- The antibacterial agents used for blind drug therapy include:
 - For Gram-positive bacteria – Penicillin, cloxacillin, fusidic acid, vancomycin, etc.
 - For Gram-negative bacteria – Ampicillin, gentamicin, carbenicillin, tobramycin, azlocillin, cefotaxime, etc.
 - Metronidazole for anaerobes

86

Chapter

Pyrexia of Unknown Origin (PUO)

■ Explain 'pyrexia of unknown origin' (PUO).

The normal body temperature is 37°C (98.8°F). The elevation of body temperature above the normal is considered as fever. When fever persists or lasts for 10 days or more without any obvious cause then it is termed PUO or fever of unknown origin (FUO).

■ What are the common causes of PUO?

Causes of PUO can broadly be categorized into two groups:

1. Infectious causes
2. Noninfectious causes

Infectious Causes

Bacterial Causes

- *Mycobacterium tuberculosis*
- *Salmonella* spp.
- *Brucella* spp.
- *Chlamydia psittaci*
- *Rickettsia* spp.
- *Francisella tularensis*
- Streptococci
- Pneumococci
- *Bacteroides* spp.
- *Treponema pallidum*
- Atypical mycobacteria
- *Coxiella burnetii*

Viral Causes

- Cytomegalovirus
- Epstein-Barr virus
- Hepatitis A and B viruses
- Arboviruses
- Adenoviruses
- Myxoviruses
- Human immunodeficiency virus (HIV)
- Haemorrhagic fever viruses

Fungal Causes

- *Candida albicans*
- *Cryptococcus neoformans*
- *Histoplasma capsulatum*
- *Aspergillus* spp.

Parasitic Causes

- Malarial parasites
- *Leishmania* spp.
- *Toxoplasma gondii*
- *Schistosoma* spp.
- Filarial parasites—*Wuchereria bancrofti*, *Brugia malayi* and *Onchocerca volvulus*

Noninfectious Causes

- Neoplasms such as lymphoma, leukaemia, hepatoma
- Collagen diseases - SLE, rheumatic fever, etc.
- Metabolic disorders such as gout and porphyria
- Anaemia
- CNS abnormalities
- Granulomatous diseases- sarcoidosis, Crohn's disease
- Drug induced fever, e.g. sulphonamide reaction
- Allergic reactions such as serum sickness
- Alcoholic patient with liver disease
- Trauma such as crush injury, head injury
- Vascular diseases such as arteritis, cerebrovascular accidents, myocardial infarction, pulmonary thromboembolism
- Miscellaneous causes—dehydration, hypothalamic lesions, thyroiditis, etc.
- Heat fever—during summer days body temperature may become elevated towards early afternoon
- Psychogenic fever, i.e. fictitious fever

■ Discuss the pathogenesis of PUO.

Regulation of body temperature is a function of thermoregulatory centre in the hypothalamus. This centre consists of pyrogen-sensitive neurons, which are responsible for control of body temperature.

Pyrogens

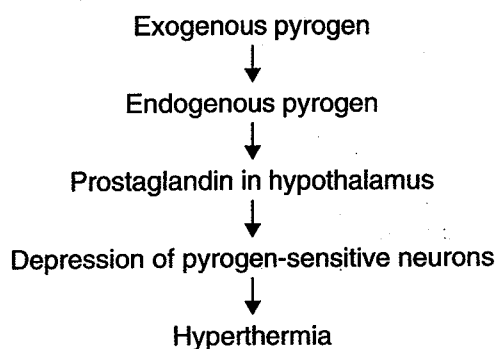
The agents that produce fever are called pyrogens. Pyrogens are of following two types:

1. Exogenous pyrogens
2. Endogenous pyrogens

Exogenous pyrogens are the product released from microorganisms, which interact with host cell and release endogenous pyrogen that stimulates the thermoregulatory centre in the hypothalamus resulting in fever.

Endogenous pyrogens are products released from monocytes, macrophages, neutrophils, eosinophils, certain tumour cells, etc. which induce fever. Initially they were called by different names, but recently the name interleukin-1 is proposed for these molecules.

The interleukin-1 via blood enters into hypothalamus and induces formation of prostaglandins. The function of specific neurons in hypothalamus is depressed by prostaglandins, resulting in hyperthermia. The process is summarized in Flowchart 86.1.



Flowchart 86.1 Pathogenesis of PUO.

■ State the clinical features of PUO.

Clinical Features

- In persons with acute febrile illness (microbial infection), the symptoms are:
 - Chills followed by rapid rise of temperature
 - Flushing of tissue
 - Intense sweating
 - Malaise
 - Lethargy
 - Weakness
 - Shock

In persons with neoplastic disease, the symptoms are:

- Emaciation
- Fever
- Chills
- Weight loss
- Headache
- Photophobia
- General malaise

Systemic symptoms in fever are:

- Pain in back
- Generalized myalgias
- Arthralgia without arthritis
- Sometimes delirium and convulsions

■ Describe the methods used in laboratory diagnosis of PUO.

The following methods are used for diagnosing PUO:

Haematological Investigations

These include the following tests:

- Haemoglobin estimation to rule out anaemia
- WBC counts:
 - Neutrophilia suggests bacterial infection
 - Atypical mononuclear cells suggests glandular fever
 - Eosinophilia suggests parasitic infections
- Peripheral smear (thick and thin) to rule out parasitic infections such as malaria, leishmaniasis, trypanosomiasis, filariasis, etc.
- Erythrocyte sedimentation rate to rule out tuberculosis, malignancy, collagen diseases, etc.

Biochemical Investigations

These include liver function tests such as:

- Aminotransferase—in viral hepatitis
- Alkaline phosphatase—in brucellosis
- alpha - fetoprotein in hepatoma

Microbiological Investigations

1. Blood Culture

Repeated blood cultures are done, as bacteria may not be continuously present in the blood.

2. Urine Examination

- Routine microscopy—pus cells
- Z-N stain—to rule out tuberculosis
- Routine culture—for bacterial infection
- Culture on L-J for tubercle bacilli

3. Stool Examination

- Stool microscopy—for detecting ova and cyst
- Stool culture—to rule out GIT infections

4. Sputum Examination

- Z-N stain—to rule out tuberculosis
- Routine culture and culture on L-J medium to rule out bacterial and mycobacterial causes

Examination of Body Fluids

Depending upon the suspected illness, various body fluids such as CSF, pleural fluid, bone marrow, bile, etc. can be examined microscopically and by culture for different pathogens.

- Anaerobic culture of pus and other specimens to rule out anaerobic infections
- Fungal culture to rule out fungal causes of PUO
- Viral and parasitic cultures are difficult and routinely impracticable

Serological Tests

- Serological tests are important in the diagnosis of viral infections, collagen disorders and also in some bacterial, fungal and protozoal infections
- The serological tests, which are commonly used in PUO are, as follows:
 - Widal test—for enteric fever
 - VDRL test—for syphilis
 - Weil-Felix test—for rickettsial infections
 - *Brucella* agglutination test and complement fixation test (CFT)—for brucellosis
 - Paul-Bunnell test—for infectious mononucleosis
 - ASO test—for streptococci
 - RA test—for rheumatoid arthritis
 - Neutralization test—for viral infections
 - ELISA and Western-blot test—for AIDS
 - ELISA, RIA, indirect immunofluorescence tests, CFT, etc.—used in various infectious and noninfectious diseases

Skin Test

In sub-acute and chronic infections, the results of skin test help in confirming or excluding diagnosis. The diseases included are tuberculosis, histoplasmosis, tularaemia, coccidioidomycosis, sarcoidosis, etc.

Animal Inoculation

Animal experiments can be performed in infections such as leptospirosis, brucellosis, rickettsial infections, etc.

Histopathologic Examination

Biopsy is the best method for definitive diagnosis. Histologic examination of tissue helps in the definitive diagnosis of neoplastic diseases, infectious diseases and granulomatous diseases.

Radiological Investigations

Radiography is helpful in the diagnosis of certain cases of PUO.

Examples

1. Chest X-ray—in tuberculosis
2. Dental and sinus X-ray—in sinusitis, dental sepsis
3. Barium studies—in inflammatory bowel disease
4. Intravenous pyelogram (IVP)—to rule out pyelonephritis, perinephric abscess
5. Ultrasound—ultrasonography for imaging abdominal or pelvic abscess

Scanning

- Radio-nucleotide scanning helps in definitive diagnosis and in some diseases it is the only procedure by which diagnosis can be made
- Examples of diseases in which scanning is required for diagnosis are:
 - Liver scan—for liver abscess or hepatoma
 - Whole body (CAT) scan—for cryptic abscess, neoplasms
 - Gallium scan—to locate pyogenic infection

Magnetic resonance imaging (MRI)

Helps in definitive diagnosis of many diseases.

Laparotomy

Exploratory surgery is helpful in the diagnosis of neoplastic diseases, infections diseases and granulomatous diseases. It is a last resort but scanning has now restricted its indications.

■ How can PUO be treated?

PUO can be treated in the following ways:

- Antipyretics—to block the synthesis of prostaglandin, which is responsible for induction of fever
- Antibiotic therapy in identified cases, also in unidentified cases based on clinical presentation (blind drug therapy)
- Specific antimicrobial therapy in identified and unidentified cases of tuberculosis, amoebiasis, malaria, etc.
- Corticosteroids in collagen disorders
- Cooler surroundings for heat fever

87

Chapter

Meningitis

■ Define meningitis.

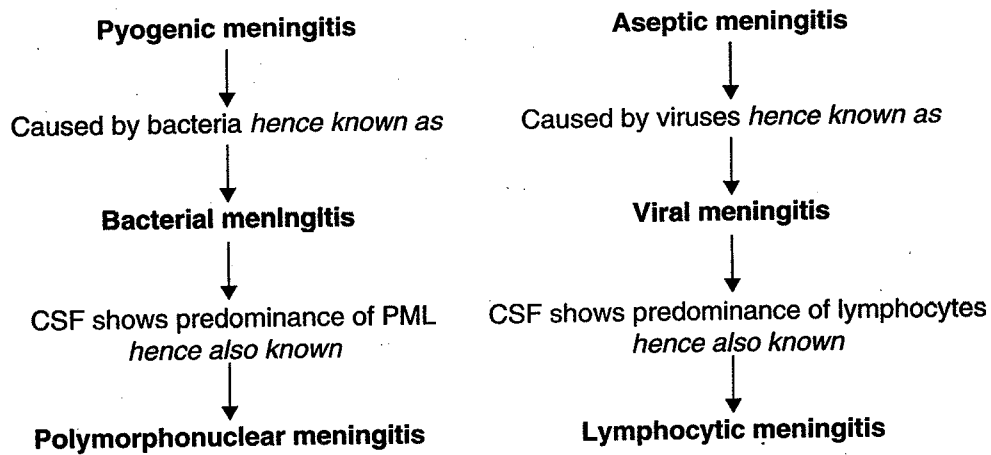
Meningitis is defined as infection of the meninges, the membranous covering of the brain and spinal cord. The infection results in inflammation of the meninges (pia-arachnoid).

■ What is encephalitis?

Encephalitis is invasion of the brain tissue and is often accompanied by inflammation of the meninges (meningoencephalitis).

■ Describe the different types of meningitis.

Meningitis is of two types: pyogenic meningitis and aseptic meningitis; they are also known by other names depending on the aetiological agents and the effect produced by them (Flowchart 87.1).



Flowchart 87.1 Types of meningitis.

■ Mention the most common agents causing meningitis.

Meningitis causing agents are as follows:

Bacterial Agents

Primary pathogens

- *Neisseria meningitidis*
- *Haemophilus influenzae*
- *Diplococcus pneumoniae*

Other bacterial pathogens

- *Staphylococcus aureus*
- *Escherichia coli*
- *Klebsiella* spp.
- *Brucella* spp.
- Beta-haemolytic streptococcus Group B
- *Pseudomonas aeruginosa*
- *Salmonella* spp.
- *Staph. epidermidis*
- *Proteus mirabilis*
- *Mycobacterium tuberculosis*

Viral Agents

- Enteroviruses
- Paramyxoviruses
- Herpes viruses
- Adenoviruses
- Arboviruses

Fungal Agents

- *Cryptococcus neoformans*
- *Candida albicans*
- *Histoplasma capsulatum*
- *Blastomyces dermatitidis*

Parasitic Agents

- *Entamoeba histolytica*
- *Naegleria* spp.
- *Acanthamoeba* spp.
- *Toxoplasma gondii*

■ Describe the pathogenesis of meningitis.

Spread of infection to the CNS occurs in the following ways:

- **Haematogenous route:** The aetiological agent invades the mucous membrane of nasopharynx or oropharynx and reaches the bloodstream and it is disseminated during the course of bacteraemia or viraemia. Sometimes, the primary involvement may be pneumonia, endocarditis, osteomyelitis, etc.
- **Direct spread:** Spreading of organisms to CNS also occurs directly through sinuses from a local focus of infection. Direct spread from middle ear (otitis media, mastoiditis), sinuses (sinusitis), nose and nasopharynx may occur
- **Invasion via nerves:** Viruses may also travel along the nerves

■ Describe the clinical features of meningitis.**Common Clinical Features of Meningitis**

- Fever with chills
- Severe headache with malaise
- Nausea and vomiting
- Photophobia
- Convulsions

- Irritability, apathy or drowsiness progressing to unconsciousness
- Altered mental state
- Signs of meningeal irritation—neck and spinal stiffness
- Coma
- Death

Neonatal meningitis—the characteristic features are not seen. Features indicative of neonatal meningitis are as follows:

- Baby is unwell
- Failure to feed
- Frequent vomiting

Complications

- Disseminated intravascular coagulation
- Shock
- Acute renal failure

■ How can diagnosis of meningitis be made in a laboratory?

Collection of Specimens

CSF is collected in a sterile container by lumbar puncture under aseptic conditions before the institution of antibiotics. Preferably, CSF should be collected in three different tubes, one for cell count, one for biochemical examination and one for culture.

Transport of CSF

- Should be transported immediately to the laboratory without delay.
- If delay is expected, especially in case of CSF subjected to microbiological investigations, it should be incubated or left at room temperature.
- If delay is unavoidable, CSF should be collected in a transport medium such as 1% glucose broth, Stuart's transport medium or Amies medium.

Processing of CSF

In the laboratory, the CSF is centrifuged at $1500 \times g$ for 15 minutes. The supernatant fluid is removed and used for biochemical and serological studies and sediment is used for culture and smear studies.

Rapid Diagnostic Methods

- **Direct wet preparation:** Wet mount of CSF is observed for cytological studies, which show
 - Predominance of neutrophils in pyogenic meningitis
 - Predominance of lymphocytes in viral meningitis
 - 90% lymphocytes and 10% neutrophils in tuberculous meningitis
- **India ink preparation:** used for demonstration of capsule in *Cryptococcus neoformans* and also for demonstration of capsulated bacteria
- **Quellung reaction:** used for identification of *H. influenzae* type b, meningococci and pneumococci
- **Gram stain:** Gram-stained smear of CSF sediment for demonstration of bacteria and cells
- **Ziehl-Neelsen stain:** used for demonstration of *M. tuberculosis* if tuberculous meningitis is suspected
- **Direct immunofluorescence test:** detects Ags by using specific Ab and fluorescent dye
- **Counter current immunoelectrophoresis:** It is used for detection of soluble antigens of meningococci, pneumococci, Group B streptococci, *H. influenzae*, *E. coli*, *Listeria monocytogenes*, etc. in CSF

- **Latex agglutination test:** It is for detection of antigen in CSF in pneumococcal, meningococcal, Group B streptococcal and *H. influenzae* meningitis
- **Coagglutination test:** It is used for detecting antigen in CSF in meningitis caused by *H. influenzae*, pneumococci, meningococci, *Salmonella* spp., streptococci, etc.
- **Limulus amoebocyte lysate assay test:** used for assay of endotoxin of Gram-negative bacteria by testing for coagulation of an extract prepared from amoebocytes (blood cells of horse shoe crab)
- **ELISA:** used for detection of antigen

Culture

- A loopful of centrifuged deposit is inoculated on
 - Blood agar
 - Chocolate agar
 - MacConkey's agar
 - Thioglycollate broth
 - If indicated, Lowenstein-Jensen (L-J) medium and tryptic soya broth
- A part of CSF is mixed with equal volume of glucose broth and incubated for extended culture
- One blood agar plate is incubated anaerobically; another blood agar plate and chocolate agar plate are incubated in candle jar (5–20% CO₂). Another blood agar plate and other media are incubated aerobically at 37°C. The media should be incubated at least for 72 hours. After incubation, plates are observed for growth, colony morphology is studied; each different type of colony is reported and identified using standard biochemical and serological procedures
- Blood culture is also useful in meningitis and is positive in about 40% cases of bacterial meningitis
- Study of antibiotic sensitivity pattern by Kirby-Bauer's disc diffusion method or Stoke's method is necessary as sensitivity pattern is not predictable except for a few organisms

Fungal culture

If direct microscopic examination shows fungi, then CSF is inoculated on media such as Sabouraud's dextrose agar, brain-heart infusion agar or other suitable media. The CSF is inoculated on agar plate or slant and incubated at room temperature for 4 weeks and another set at 35°C. After appropriate incubation period, it is observed for growth, and positive cultures are identified using standard techniques.

■ How would you treat meningitis?

Meningitis can be treated in the following ways:

- Early parenteral antibiotics in high doses. Initial treatment by intramuscular or intravenous route. Later on antibiotics can be given orally. This therapy should be directed by local antimicrobial sensitivity pattern. The antibiotics used are benzyl penicillin, chloramphenicol, beta-lactamase stable cephalosporin, sulphonamide, etc. These can be used in different combinations
- *Tuberculous meningitis*—antituberculous triple therapy (rifampicin, isoniazid and pyrazinamide) for one year
- *Neonatal meningitis*—mostly caused by coliform bacteria. Antibiotics that can be used are gentamicin and ampicillin or chloramphenicol or cefotaxime. For group B streptococci meningitis penicillin and gentamicin can be used
- *Viral meningitis*—no specific antiviral therapy is available. Acyclovir can be used in Herpes simplex virus

88

Chapter

Respiratory Tract Infections (RTI)

■ What are respiratory tract infections? Comment on their spread.

Respiratory tract infections (RTI) include infections of upper respiratory tract (Infections of ear and sinuses, and throat and pharynx) and lower respiratory tract (Infections of trachea and bronchi). RTI are the most commonly encountered diseases. They are very important cause of sickness and account for 50% cases of general practitioner's consultations.

- Infection is air borne, i.e. occurs by inhalation
- Talking, coughing and sneezing spread the infection
- Sneezing is most dangerous and can expel about 10,000–100,000 organisms/sneeze
- A considerable number of organisms are also expelled during coughing and talking. Each infectious droplet expelled out contains one or two microorganisms
- Air is a potential source of infectious agents for respiratory infections. Close contact with an infected person in school, at work and while socializing allows transfer of causative agents. The infections are common during winter season, i.e. between October and March

■ Enumerate the most common aetiological agents of respiratory tract infections.

Agents responsible for causing respiratory tract infections are as follows:

Bacterial Agents

- *Streptococcus pyogenes*
- *Corynebacterium diphtheriae*
- *Mycoplasma pneumoniae*
- *Borrelia vincentii*
- *Staphylococcus aureus*
- *Haemophilus influenzae*
- Pneumococci
- *Mycobacterium tuberculosis*
- Atypical mycobacteria
- Group B streptococci
- *Pseudomonas aeruginosa*
- *Bordetella pertussis*
- *Legionella pneumophila*
- *Neisseria meningitidis*
- *Klebsiella* spp.
- *Streptococcus viridans*

Viral Agents

- Rhinoviruses
- Influenza A and B viruses

- Adenoviruses
- Respiratory syncytial virus
- Parainfluenza viruses
- Measles virus
- Coronaviruses
- Varicella-zoster virus

Fungal Agents

- *Candida albicans*
- *Aspergillus* spp.
- *Cryptococcus neoformans*
- *Histoplasma capsulatum*
- *Blastomyces dermatitidis*
- *Coccidioides immitis*
- *Pneumocystis carinii*

■ Briefly describe the pathogenesis of respiratory tract infections.

Development of respiratory tract infections takes the following course:

- Source of infection—is patient and carrier
- Mode of transmission—is through inhalation of aerosols
- Portal of entry—is respiratory tract
- After gaining entry into susceptible host, the causative agent adheres to mucosa of respiratory tract with the help of pili or other adherence factors. By resisting the local defenses, it starts multiplication resulting in colonization. Once the organism colonizes, with the help of various virulence factors, clinical symptoms are produced

■ What are the clinical features of respiratory tract infections?

Respiratory tract infections include:

- | | |
|---------------------------------------|-------------|
| • Infection of middle ear and sinuses | } Upper RTI |
| • Infection of throat and pharynx | |
| • Infection of trachea and bronchi | } Lower RTI |
| • Infection of lungs | |

Upper Respiratory Tract Infections

Infection of Ear and Sinuses

- Acute otitis media
- Otitis externa
- Acute sinusitis

Infections of Throat and Pharynx

- Tonsillitis
- Pharyngitis
- Sore throat
- Laryngitis
- Epiglottitis
- Peritonsillar abscess
- Oral thrush
- Vincent's angina

Lower Respiratory Tract Infections

Infections of Trachea and Bronchi

- Bronchitis
- Bronchiolitis
- Bronchiectasis
- Tracheitis
- Tracheobronchitis

Infections of Lungs

- Pneumonia
- Lung abscess
- Empyema

Respiratory tract infections may lead to septicaemia and bacteraemia.

■ Explain sore throat. Comment on its causes and pathogenesis.

Sore throat is a condition where the mucus membrane in the throat is inflamed because of an infection. It is the most common disease in young children caused by bacteria, viruses and fungi.

Causes

Sore throat is caused by the following agents:

Bacterial Agents

- *Streptococcus pyogenes* (Group A streptococcus)
- *Corynebacterium diphtheriae*
- Beta-haemolytic streptococci—Group C and G
- *Staphylococcus aureus*
- *Borrelia vincentii*
- *Fusobacterium* spp.
- *Neisseria gonorrhoeae*
- *Mycoplasma pneumoniae*

Viral Agents

- Adenoviruses
- Influenza viruses
- Parainfluenza viruses
- Coxsackie viruses
- Rhinoviruses
- Respiratory syncytial virus
- Coronavirus

Fungal Agent

Candida albicans

Pathogenesis

Infection occurs by

- Droplet inhalation
- Portal of entry is respiratory tract

■ Mention the clinical features of sore throat.

The incubation period of sore throat is 1–3 days. The main presenting features are as follows:

- Fever
- Pain on swallowing
- Tonsillitis
- Pharyngitis
- Dysphagia
- Painful cervical lymphadenopathy

■ Explain pneumonia. Which microbial agents are common cause of pneumonia?

Pneumonia is inflammation of the lung with production of alveolar exudates. The inflammation and consolidation of the lung is caused by microorganisms. Microbial agents causing pneumonia are as follows:

Bacterial Agents

- *Streptococcus pneumoniae*
- *Staphylococcus aureus*
- *Streptococcus pyogenes*
- *Klebsiella pneumoniae*
- *Neisseria meningitidis*
- *Pseudomonas aeruginosa*
- *Haemophilus influenzae*
- *Legionella pneumophila*
- *Mycoplasma pneumoniae*
- *Chlamydia pneumoniae*

Viral Agents

- Influenza A and B viruses
- Respiratory syncytial virus
- Adenovirus
- Parainfluenza virus
- Rhinovirus
- Coronaviruses
- Coxsackie viruses
- Varicella-zoster virus

Fungal Agents

- *Cryptococcus neoformans*
- *Histoplasma capsulatum*
- *Aspergillus* spp.
- *Blastomyces dermatitidis*
- *Candida albicans*
- *Pneumocystis carinii*

■ How is pneumonia transmitted?

Pneumonia occurs by:

- Droplet inhalation
- Aspiration of upper respiratory tract secretions containing microorganisms

- Haematogenous or lymphatic dissemination
- Direct contact with respiratory secretions

■ Describe the clinical features of pneumonia.

Characteristic Symptoms of Pneumonia

- Fever and chills
- Pleuritic chest pain
- Cough—initially nonproductive, subsequently becomes productive, i.e. mucopurulent yellow or green sputum is produced. Mucoïd sputum may be blood tinged (atypical pneumonia)
- Headache
- Malaise
- Generalized myalgia
- Nausea and vomiting
- Pharyngitis and sore throat
- Rhinorrhoea
- Coryza
- Cyanosis

Clinical Types

- **Lobar pneumonia:** It is an acute inflammation caused by homogenous consolidation of one or more lobes or segments of lungs. It is most commonly caused by *Str. pneumoniae*
- **Bronchopneumonia:** It is a secondary pneumonia invariably preceded by bronchial infection. It is an acute inflammation of bronchi, especially the terminal bronchioles, which are filled with pus. It is commonly caused by *Str. pneumoniae* and *H. influenzae* and rarely by *Staph. aureus* and coliforms
- **Atypical Pneumonia:** In this type, there is a patchy consolidation of lungs and it is progression of upper respiratory tract infection. The causative agents are *M. pneumoniae*, *Coxiella burnetii* and *Chlamydia psittaci*

■ Describe the laboratory diagnosis of respiratory tract infections.

Collection of Specimens

- Throat swab, ear swab, nasal swab, etc. are collected with the help of cotton swab
- In case of lower respiratory tract infections, sputum is the most commonly collected specimen
- The sputum should be collected in a sterile container with an attempt to minimize contamination with saliva
- Early morning sputum is more purulent—hence preferred
- Other specimens collected in lower respiratory tract infections are:
 - Transtracheal aspirates
 - Bronchial aspirates
 - Pleural fluid
 - Transbronchoscopic lung biopsy—specimens collected using invasive techniques
 - Blood for culture is collected in pneumonia

Transport of Specimens

In case of upper respiratory tract infections where specimen is collected using swab, it should be transported immediately to laboratory without delay to avoid death of delicate pathogens because of drying or desiccation.

- Immediate transfer of specimen, including sputum, also avoids overgrowth of robust bacteria like coliforms and avoids death of delicate organisms like *H. influenzae*

- If delay is expected then specimen should be collected in a suitable transport media such as:
 - Modified Stuart's transport medium
 - Cary and Blair's transport medium
 - Amies medium or
 - Ringer's solution to keep the swab moist
- An alternative is to hold specimen at 4°C during the transport

Processing of Specimen

Direct Examination

- **Gram Stain**—for demonstration of bacteria and fungi. Particularly, helpful in oral thrush and Vincent's angina
- **Ziehl-Neelsen stain**—for demonstration of mycobacteria for presumptive diagnosis of tuberculosis and atypical mycobacterial infections. Auramine stain can also be used
- **Potassium hydroxide preparation (KOH mount)**—use of 10% KOH mount for demonstration of fungi
- **Negative stain** for demonstration of capsule, e.g. pneumococci
- **Direct wet mount and silver methenamine stain** for *Pneumocystis carinii*
- **Direct fluorescent antibody test** for demonstration of antigen in specimen
- **Electron microscopy** for demonstration of Chlamydia and viruses

Culture

- The specimen is inoculated on chocolate agar, sheep blood agar, MacConkey's agar and other selective media as follows:
 - Loeffler's serum slope } *Corynebacterium diphtheriae*
 - Potassium tellurite agar }
 - L-J medium—*M. tuberculosis*
 - Bordet-Gengou medium—*Bordetella* spp.
 - Neomycin blood agar—anaerobes
 - Sabouraud's dextrose agar—fungi
 - Tissue culture } For viruses and *Chlamydia*
 - Chick embryo }
- Blood agar and chocolate agar plates are incubated under 5–10% CO₂ for culture of micro-aerophilic organisms
- Another blood agar and neomycin blood agar plates are incubated anaerobically for culture of anaerobes
- Another blood agar and other media are incubated under aerobic conditions
- After incubation, growth is studied and identified using conventional techniques

Ag Detection

- Detection of antigen in specimen: Capsular antigen of pneumococci can be detected by quellung reaction, counter current immunoelectrophoresis test and latex agglutination test
- *H. influenzae* and streptococcal antigens can be detected by immunoelectrophoresis (IEP), latex agglutination test and coagglutination test
- *Legionella* Ag can be detected by radioimmunoassay

Ab Detection

- ELISA for detection of *B. pertussis*
- **Complement fixation test** for *Mycoplasma*, *Coxiella*, *Chlamydia* spp. and *Bordetella* spp.
- **Indirect immunofluorescence test** for *Bordetella*, *Legionella* spp., pneumococci, *T. pallidum*.

- **Antistreptolysin O (ASO)** for streptococci
- **C-reactive protein test (CRP)** for pneumococci
- **Indirect haemagglutination test** for pneumococci

Serology

Serological tests such as neutralization test, CFT, ELISA, RIA, CIEP and indirect immunofluorescence test, agglutination test, latex agglutination test, immunoperoxidase test, etc. are used in the diagnosis of RTI caused by viruses.

Other Techniques

Newer techniques such as polymerase chain reaction or dot-blot hybridization can also be used for diagnosis of RTI.

■ **Suggest treatment for respiratory tract infections.**

Respiratory tract infections are treated on following lines:

- Antibiotics are used to eliminate bacteria from respiratory tract and also to treat secondary infections in viral infections of respiratory tract
- Before the availability of sensitivity report, the antibacterial agents are used. These are, as follows:
 - Ampicillin
 - Amoxycillin
 - Co-trimoxazole
 - Erythromycin
 - Penicillin
 - Metronidazole for anaerobes
- Correct antibiotic treatment is instituted after the availability of sensitivity report
- Antituberculous drugs are used for *M. tuberculosis*
- Antifungal agents are used for treating mycotic infections
- Viral infections are self-limited, hence no specific treatment is required

89

Chapter

Diarrhoeal Diseases

■ **Define the following terms: (a) Diarrhoea, (b) Gastroenteritis, (c) Dysentery, (e) Traveller's diarrhoea, and (f) Food poisoning.**

- (a) **Diarrhoea** may be defined as an increase in the frequency, fluidity or volume of bowel movements relative to the usual habits of each individual. As a rough guide, passage of three or more motions a day can be taken as diarrhoea
- (b) **Gastroenteritis** may be defined as inflammation of the mucus membrane of the stomach and intestine resulting in frequent loose motions with or without blood and mucous, pain in abdomen and may be associated with vomiting
- (c) **Dysentery** means presence of blood and mucous in stool, often with tenesmus
- (d) **Traveller's diarrhoea** is an acute diarrhoeal illness that sometimes occurs in visitors from foreign countries within a week or two in a developing country
- (e) **Food poisoning** is an acute diarrhoea with or without vomiting caused by microbial contamination of food

■ **Enumerate the most common aetiological agents that cause diarrhoea.**

Bacterial Agents

- *Vibrio cholerae*—classical and El Tor
- Noncholera vibrio (NAG vibrio)
- *Vibrio parahaemolyticus*
- Enterotoxigenic *Escherichia coli* (ETEC)
- Enteropathogenic *Escherichia coli* (EPEC)
- Enteroinvasive *Escherichia coli* (EIEC)
- Enterohaemorrhagic *Escherichia coli* (EHEC)
- *Salmonella* spp.
- *Shigella* spp. (all four species)
- *Clostridium welchii*
- *Cl. botulinum*
- *Cl. difficile*
- *Bacillus cereus*
- *Staphylococcus aureus*

Viral Agents

- Rotavirus
- Norwalk virus
- Adenoviruses
- Calciviruses
- Coronaviruses
- Astroviruses
- Enteroviruses

Parasitic Agents

- *Entamoeba histolytica*
- *Giardia lamblia*
- *Balantidium coli*
- *Cryptosporidium* spp.
- *Isospora belli*

Fungal Agent

Candida albicans.

■ Discuss the pathogenesis of diarrhoeal diseases.

- Infection occurs by ingestion of contaminated food or water. Contaminated hands can also transmit infection, e.g. dysentery, in which direct hand to mouth transmission occurs through contaminated fingers while feeding (nurses may transmit diarrhoea to infants). The infection can be acquired from a single common source resulting in large-scale outbreaks
- The pathogenic mechanisms resulting in diarrhoea are as follows:

1. Toxin Type (Noninvasive Type)

- The toxin produced by causative agent affects fluid secretion, cell function or neurological function. Different types of toxins contribute to the pathogenesis of diarrhoea in various ways:
 - Cytotoxins act on intestinal epithelial cells and destroy them affecting secretory or absorptive functions of cells
 - Enterotoxins cause an alteration in the metabolic activity of intestinal epithelial cells, resulting in outpouring of electrolytes and fluids into the lumen.

2. Growth Within or Close to Intestinal Mucosal Cells

Multiplication of organisms within certain cells of mucosa cause destruction of cell function. Viruses such as rotavirus and adenoviruses multiply within certain cells of the small intestine thereby disrupting normal functioning and causing diarrhoea.

3. Invasion of the Mucosal Epithelium Causing Cellular Destruction

Invasive microorganisms such as *Salmonella* spp., *Shigella* spp., enteroinvasive *E. coli*; and parasites such as *Entamoeba histolytica*, *Balantidium coli*, invade the intestinal epithelial cells and produce the symptoms of dysentery.

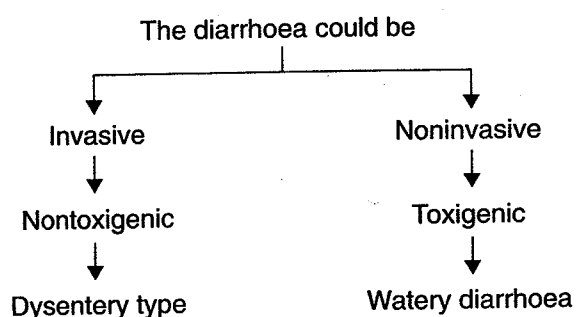
4. Adhesion to Intestinal Mucosa, Interfering Absorption and Secretion

Microorganisms such as *Giardia lamblia* and enteroadherent *E. coli* adhere to the mucosa in the small intestine and destroy the ability of the mucosal cells to participate in normal function of secretion and absorption and produce diarrhoeal illness.

■ Mention the clinical features of diarrhoeal diseases.

The infection effecting diarrhoea can be categorized into two types, invasive and noninvasive, leading to different types of diarrhoeal diseases (Flowchart 89.1).

- **Invasive diarrhoea** is characterized by
 - Mild fever
 - Abdominal pain
 - Passage of bloody mucoid stool
 - Vomiting



Flowchart 89.1 Types of diarrhoeal diseases.

- **Noninvasive diarrhoea** is characterized by
 - Fever, low or absent
 - Watery diarrhoea—no blood and mucus in stool
 - Profuse secretion of fluid and electrolytes
 - Nausea and vomiting

■ Describe the laboratory diagnosis of diarrhoeal diseases.

Collection of Specimen

- Stool sample—should be collected in a clean sterile container
- Rectal swabs—collected if faeces are not readily obtainable
- Vomitus—is less useful

Transport of Specimen

- The specimen should be transported immediately. If delay of more than two hours is expected in case of stool for bacterial culture, the specimen should be collected in suitable transport media such as:
 - Cary–Blair transport medium
 - Stuart's transport medium
 - 0.33 M phosphate buffer mixed with equal parts of glycerol (pH 7.0)

Microscopic Examination

Various microscopic methods are available for detecting microorganisms. These are, as follows:

- **Gram stain** is useful in staphylococcal enterocolitis, cholera (coma-shaped bacteria) and *C. difficile*—antibiotic-associated enterocolitis
- **Wet mount** for pus cells and RBCs which indicate invasive disease
- **Saline and iodine preparation** for trophozoite and cyst of protozoal parasites
- **Hanging drop preparation** for darting type of motility of vibrios
- **Modified acid fast stain** for *Cryptosporidium* spp. and *Isospora* spp.
- **Electron microscopy and immune-electron microscopy** for viruses (Rotavirus and Norwalk virus)
- **Direct immunofluorescence test** for demonstration of various organisms causing diarrhoea. A monoclonal antibody fluorescent stain is used to visualize *Giardia* and *Cryptosporidium*

Culture

- To identify and characterize the bacterial pathogen, the specimen is inoculated on blood agar, MacConkey's agar and various selective media to inhibit the growth of normal flora
- For culture of virus—tissue cultures are used
- For *Candida albicans*—stool is inoculated on Sabouraud's dextrose agar
- For parasites—cultures are routinely impracticable

Identification

The organisms grown in culture are identified by using standard biochemical reactions and serological tests.

Immunological Tests

Various immunological tests are used for detecting antigen in faeces. These include:

- **Latex agglutination test**—for detection of rotavirus and cholera toxin
- **Coagglutination test**—for labile toxin of *E. coli* and cholera toxin
- **Agar gel diffusion** (Biken test)—for labile toxin (LT) of *E. coli*
- **ELISA**—to detect antigens of rotavirus, *G. lamblia*, cholera toxin, *E. coli* LT and ST and other agents

Other Tests: such as DNA probes and PCR

■ How can diarrhoeal diseases be treated?

For diarrhoeal diseases, following treatment measures are adopted:

- Major objectives in the management are early replacement of water and electrolytes to prevent or treat dehydration and maintenance of adequate nutrition. Specific treatment is not needed except for protozoal diarrhoea
 - WHO introduced oral rehydration fluid containing

Sodium chloride	3.5 gm
Sodium bicarbonate	2.5 gm
Potassium chloride	1.5 gm
Glucose	20 gm
Water	1000 ml
 - This fluid compensates with electrolyte loss in diarrhoea and helps to control diarrhoea. Intravenous fluids are needed in severe cases of dehydration
- Routine antibiotic therapy has no place in the management of uncomplicated cases. Antibiotics are indicated in complicated cases with systemic symptoms such as fever, toxicity or shock

■ What is food poisoning?

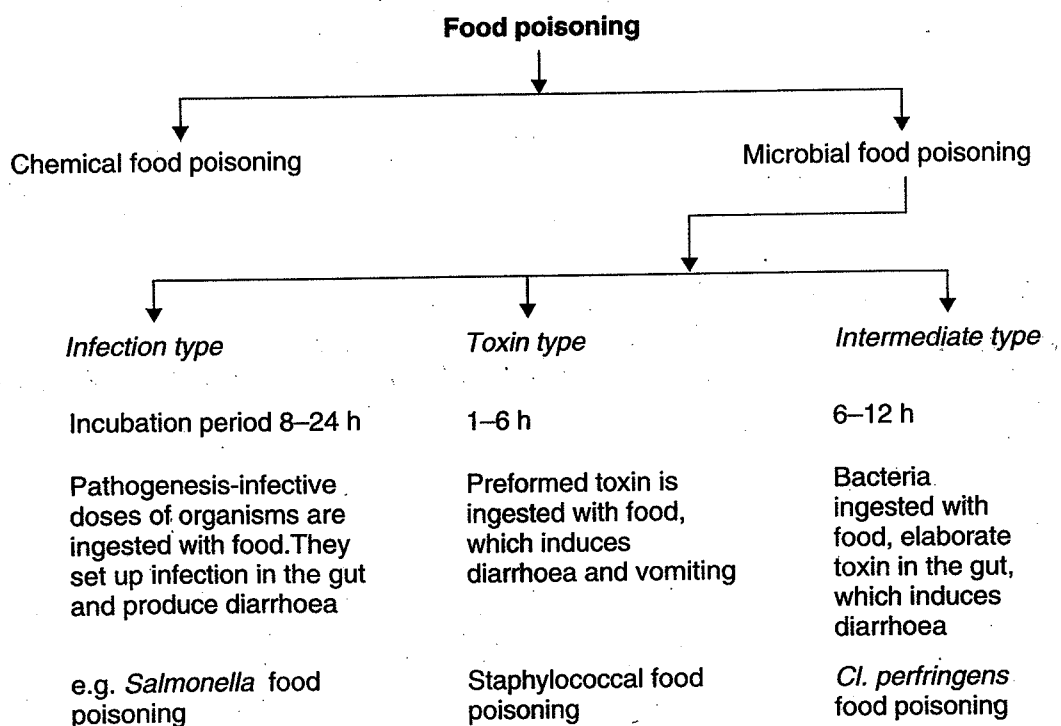
Food poisoning may be defined as any type of illness acquired through consumption of food or drink contaminated with microorganisms, their toxins or chemical poisons. But traditionally, it is restricted to acute diarrhoea, with or without vomiting caused by microbial contamination of food.

The term "Microbial food poisoning" has been interpreted in many ways. But it appears reasonable to restrict the definition of microbial food poisoning to diarrhoeal diseases caused by consumption of food contaminated with microorganisms or their products.

■ Classify food poisoning.

Food poisoning can be classified in two ways: (a) on the basis of mechanism of infection, and (b) on the basis of the causative agent.

Classification Based on Mechanism of Infection



Classification Based on Causative Agents

Food poisoning caused by

- Gram-positive cocci
- Gram-positive spore-forming bacilli
- Gram-negative bacilli

■ Mention the causes of food poisoning.

Causes of food poisoning are as follows:

Gram-positive Cocci

- *Staphylococcus aureus*
- *Streptococcus faecalis*

Gram-positive Spore—Forming Bacilli

- *Clostridium perfringens*
- *Cl. botulinum*
- *Bacillus cereus*

Gram-negative Bacilli

- *Salmonella* spp. – *S. typhimurium*, *S. enteritidis*, *S. thompson* (any species except *S. typhi*)
- Enteropathogenic *E. coli*
- *Vibrio parahaemolyticus*
- *V. mimicus*
- *Campylobacter jejuni*

■ Discuss the pathogenesis of food poisoning.

- Sources of infection (food vehicles) are meat, poultry, fish, eggs, salads, milk and milk products, vegetables, cereal products, boiled/fried rice, etc. (Table 89.1).
- Infection occurs by ingestion of food contaminated with bacteria or toxins. The pathogenic mechanisms are:
 - Ingestion of preformed toxin in food—organisms such as *Staph. aureus* and *B. cereus* form toxins in food. Ingestion of such food induces diarrhoea and vomiting
 - Microorganisms invading gastrointestinal tract—organisms such as *Salmonella* spp., *Campylobacter jejuni*, *Vibrio parahaemolyticus* and *V. mimicus* are ingested with food. These organisms invade the gastrointestinal tract and produce diarrhoea

Table 89.1 Causative agents and their food vehicles

Organism	Food vehicle
1. <i>Staph. aureus</i>	Meat, cream, cakes, fish, milk and milk products
2. <i>C. perfringens</i>	Meat—cold or warmed up meat dishes
3. <i>C. botulinum</i>	Canned fruit and vegetables, condiments, meat and meat products, fish and other sea foods
4. <i>B. cereus</i>	Cooked meat and vegetables, cooked rice/fried rice from Chinese restaurants
5. <i>Salmonella</i> spp.	Poultry, meat, milk, cream, eggs
6. <i>Vibrio parahaemolyticus</i>	Shell fish, marine food
7. <i>Campylobacter jejuni</i>	Unpasteurized milk

- Organisms such as *Cl. perfringens*, enteropathogenic *E. coli* and *Cl. botulinum* are ingested with food and produce toxin in the gut, which induces diarrhoea

■ What are the clinical features of food poisoning?

- Incubation period is 1–24 hours
- Food poisoning is characterized by the following symptoms:
 1. Nausea and vomiting
 2. Abdominal pain
 3. Diarrhoea
 4. Fever and rigors
 5. Muscular weakness
 6. Drowsiness
 7. Restlessness

■ What basic information is required for investigating a case of food poisoning? Mention the specimens and methods used for detecting the causative agents.

- Information helpful in investigating a case of food poisoning is as follows:
 - Clinical symptoms (sequence of symptoms)
 - Duration of symptoms
 - Persons affected (Age of infected persons and number of cases)
 - Food article consumed

This would give a clue regarding the source of infection and probable aetiological agent.

- **Specimens** used are stool, samples of suspected food, vomitus, blood—for culture and serology
- In case of death—samples of intestinal contents, spleen, liver and heart blood

Methods for Detecting Causative Agents

Microscopy

This technique is used for demonstrating the causative agent in stool and in the food consumed.

Culture

- The organism is cultured on appropriate media; both selective and nonselective media are used for isolation. Isolates are identified by standard techniques
- Repeated cultures of stool and other specimens of suspected carriers are done to trace the source of infection

Serology

It demonstrates rise in titre of Abs in the patient's serum. This method is helpful in some cases.

■ What precautions should be taken to prevent food poisoning?

Strict attention to cleanliness at all levels of preparation, storage and serving of food is the best way of preventing food poisoning.

90

Chapter

Urinary Tract Infections (UTI)

■ Define urinary tract infection.

Urinary tract infection (UTI) is defined as bacteriuria, i.e. the multiplication of bacteria in urine within the renal tract and the presence of 100,000 (10^5) per ml in the mid-stream sample of urine.

■ Describe the different types of urinary tract infections.

Urinary tract infections are infections of the kidney, ureter, bladder and urethra. They are of following two types:

1. **Upper urinary tract infection**, in which infection involves kidney or ureter only. The infections caused are:
 - Acute pyelitis—Infection of pelvis of kidney
 - Acute pyelonephritis—Infection of parenchyma of kidney
2. **Lower urinary tract infection**, in which infection is from bladder downwards. The infections caused are:
 - Urethritis—Infection of urethra
 - Cystitis—Infection of urinary bladder
 - Prostatitis—Infection of prostate

■ Mention the most common aetiological agents of urinary tract infections.

Microbes causing urinary tract infections are as follows:

Bacterial Agents

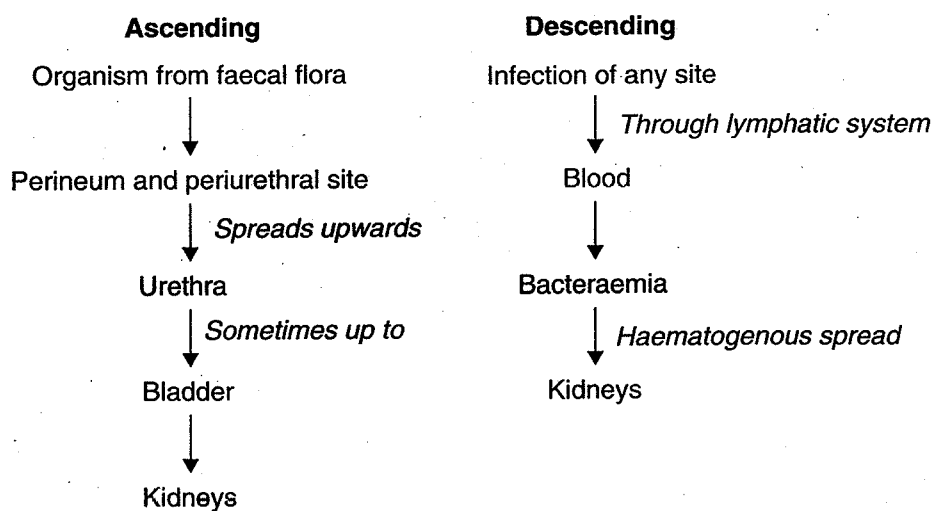
- *E. coli* (most common)
- *Proteus mirabilis*
- *Klebsiella* spp.
- *Staphylococcus saprophyticus*
- *Staph. epidermidis*
- *Staph. aureus*
- Enterococci
- *Pseudomonas aeruginosa*
- *Enterobacter* spp.
- *Citrobacter* spp.
- *Acinetobacter* spp.
- *Salmonella* spp.
- *Ureaplasma urealyticum*
- Anaerobes such as *Bacteroides fragilis*

Fungal Agents

- *Candida albicans*
- *Cryptococcus neoformans*

■ Discuss the pathogenesis of urinary tract infections.

- Adhesion of organism is an important factor in pathogenesis of UTI. The adhesion is mediated by pili or other adhesins such as colonization factor. Once it is adhered, with the help of various virulence factors, it spreads and produces pathogenic effects by resisting bactericidal and bacteriostatic effects of local tissue
- Infection may be ascending type or descending type
- The spreading of pathogenic organisms in ascending and descending type of infection is presented in Flowchart 90.1



Flowchart 90.1 Spreading of causal agent to urinary tract.

■ Mention the clinical features and complications of urinary tract infections.

Clinical Features

These are as follows:

1. Asymptomatic infection or covert bacteriuria

It is associated with active disease process in kidney. Hence, if left untreated, it can develop cystitis and might go to renal failure.

2. Symptomatic Infection

In this, UTI is associated with symptoms. The different clinical forms are:

- **Cystitis**—characterized by dysuria, frequency of micturition, fever, urgency, suprapubic pain and sometimes haematuria. More common in females because of short urethra
- **Acute urethral syndrome**—characterized by dysuria and frequency, seen in young, sexually active women
- **Pyelonephritis**—characterized by loin pain, tenderness, high fever and rigors. Chronic pyelonephritis causes general ill health and malaise with nocturia
- **Pyelitis**—a mild form of pyelonephritis with pyuria but minimal involvement of renal tissue

■ Describe the laboratory diagnosis of urinary tract infections.

Collection of Specimen

- As urethra and perineum are normally colonized, specimens of urine are frequently contaminated with normal flora from urethra and perineum
- Contamination of specimen can be prevented by using following methods of collection:

1. Suprapubic Aspiration

Collection of bladder urine eliminates urethral and vaginal contamination. This is useful in infants, small children, pregnant women and adults with full bladder.

2. Catheterization

A catheter specimen of urine is excellent but there is a risk of introducing infection. The urethral organisms may be introduced into the bladder with catheter.

3. Clean-catch Midstream Urine Collection

- It is the most convenient and most commonly used method for urine collection
- In this method, the periurethral area, (i.e. tip of penis, labial folds and vulva) is carefully cleaned with two separate washes of soap and water or mild detergent
- It is then well rinsed with sterile warm water to remove detergent
- The first portion of voiding is discarded and the subsequent midstream urine is voided directly into sterile wide-mouth container and is used for culture and other investigations
- Mid-stream urine is the most ideal specimen for the diagnosis of UTI
- For diagnosis of renal tuberculosis, early morning specimen is collected on three consecutive days in sterile containers and preserved in refrigerator till they are examined. An alternative is collection of 24 hours sample of the urine

Transport and Storage

As urine is an excellent medium for the growth of most bacteria, the specimen must be transported to the laboratory immediately to avoid multiplication of possible contaminants. If delay of more than 1–2 hours is unavoidable, one of the following methods of storage can be used.

- Refrigerate the specimen at 4°C
- Use of commercially available urine transport tube containing boric acid, glycerol-sodium formate
- Use of container with boric acid (1.8%), which is bacteriostatic

Processing of Specimen

The mere presence of bacteria in urine does not indicate urinary tract infection, the counts of 10^5 or more organisms per millilitre is the criteria. Thus, urine samples are screened for significant bacteriuria. Various screening methods are available. These include:

Microscopic Examination

- **Wet mount:** This method is used to observe pus cells, RBCs, epithelial cells and crystals. Ten or more pus cells per mm^3 of undiluted urine is an indication of significant bacteriuria
- **Gram stain:** If the Gram stained smear shows 1 or 2 bacteria per 2–3 microscopic field of uncentrifuged urine or 5 bacteria per oil immersion field of centrifuged deposit, it is considered as significant bacteriuria

Chemical Methods

Triphenyl tetrazolium chloride test (TTC): This test is based on reduction of colourless soluble chemical triphenyl tetrazolium chloride into a pink to red coloured insoluble compound, triphenyl tetrazolium formazon because of respiratory activity of growing bacteria.

Enzymatic Methods

- **Glucose oxidase test:** The test is based on utilization of small amount of glucose present in normal urine by bacteria causing UTI

- **Griess nitrate test:** The test is based on rapid reduction of nitrate to nitrite by nitrate reducing enzyme—nitrate reductase—an enzyme present in Gram-negative bacilli commonly involved in UTI
- **Leucocyte esterase test:** This is a dip stick method used for the detection of pyuria. With this test pus cell count of more than 10 per mm³ can be made
- **Catalase test:** The test is based on presence of catalase enzyme in uropathogens, which is evidenced by hydrogen peroxide. The positive test is indicated by effervescence

Culture

Culture is the most accurate and acceptable method of screening. Both quantitative and semi-quantitative methods are used.

Quantitative methods

1. Pour plate method
2. Pipette dilution method
3. Simplified spread plate method

These are the quantitative methods, which are too complicated, expensive and routinely impracticable. Hence, these are used as reference methods only.

Semiquantitative methods

1. **Calibrated loop method:** It is the most convenient method. In this method, a loop delivering 0.001 ml of urine is used to inoculate blood agar. After incubation for 18 to 24 hours, plates are examined and colonies are counted. The total number of bacteria per ml of urine is obtained as follows:
Total number of bacteria per ml of urine = No. of colonies \times 1000
This method is more suitable to study large number of urine samples
2. **Dip slide culture method:** In this method, commercially available plastic slides coated with CLED agar on one side and MacConkey's agar on other side, are inoculated by immersing into freshly passed urine or by exposing slides to stream of urine during voiding. Slides are placed in a sterile container and incubated. After incubation colonies are counted
3. **Filter paper strip technique:** In this method, the filter paper strip is dipped into urine and transferred to conventional agar plate and after incubation colonies are counted

Other screening methods

1. **Automated screening test:** Commercially available kits are used for rapid screening of urine by using light scatter photometry in 4–5 hours, e. g. Pfizer's Autobac, MS- 2/Abbot and Auto Microbic system (Vitek system)

Interpretation of Colony Count

- The term significant bacteriuria was introduced by Kass (1956). According to criteria of Kass:
 - 100,000 (10^5) or more bacteria per ml of urine indicates significant bacteriuria, sensitivity test is to be done
 - Counts between 10^4 /ml and 10^5 /ml is a doubtful significance and always a repeat culture should be done and the proper history of patient should be taken
 - Counts less than 10^4 /ml indicates no significant bacteriuria and is considered as contamination

Identification of the Bacteria

The cultures of screened positive urine samples are identified by using standard biochemical and/or serological tests.

Antimicrobial Susceptibility Testing

- Susceptibility testing is essential for choice of antimicrobial agents. Susceptibility is determined by Kirby-Bauer disc diffusion method using drugs, which are excreted in urine in high concentration. These include:
 - Ampicillin
 - Amoxycillin
 - Augmentin
 - Cotrimoxazole
 - Carbenicillin
 - Cephalexin
 - Cephaloridine
 - Ciprofloxacin
 - Gentamicin
 - Kanamycin
 - Nalidixic acid
 - Nitrofurantoin
 - Norfloxacin
 - Sulphonamides
 - Tobramycin

Other Investigations

Investigations other than culture and microscopy are used in some selective cases. These include:

1. Immunofluorescence Test

This method can be used to detect antibody-coated bacteria in urine that helps to determine whether patient is suffering from bladder infection or renal tissue infection.

2. Detection of Antibodies

Abs can be detected in serum against infecting organisms.

■ How should urinary tract infections be treated?

Treatment for urinary tract infections should be guided by susceptibility report as multiple resistance to drugs may occur in uropathogen. Sometimes, a combination of drugs is required.

91

Chapter

Sexually Transmitted Diseases (STDs)

■ Explain sexually transmitted diseases.

Sexually transmitted diseases (STDs) are a group of communicable diseases that are transmitted predominantly or entirely by sexual contact or close bodily contact with infected individuals.

These pathogens are generally present in body fluids exchanged during sexual contact. Because of their sensitivity to environmental conditions they preferably and sometimes exclusively colonize the genitourinary tract as it is moist and it is also the most protected environment for colonization.

■ Name sexually transmitted diseases along with their causal agents.

The various types of sexually transmitted diseases and the corresponding pathogens are presented in Table 91.1.

Table 91.1 Sexually transmitted diseases and their causal agents

Disease	Causal organism
Bacterial STDs	
1. Syphilis	<i>Treponema pallidum</i>
2. Gonorrhoea	<i>Neisseria gonorrhoeae</i>
3. Lymphogranulomavenereum	<i>Chlamydia trachomatis</i>
4. Nonspecific urethritis	<i>Mycoplasma genitalium</i> , <i>C. trachomatis</i>
5. Vaginitis	<i>Ureaplasma urealyticum</i> , <i>G. vaginalis</i>
6. Chancroid or soft sore	<i>Gardnerella vaginalis</i>
7. Granuloma inguinale	<i>Haemophilus ducreyi</i>
8. Neonatal sepsis	<i>Calymmatobacterium granulomatis</i>
Viral STDs	
1. Genital herpes	Group B streptococci
2. <i>Molluscum contagiosum</i>	Herpes simplex virus Types 1, 2
3. Cytomegalic inclusion	<i>Molluscum contagiosum</i>
4. Genital warts (papilloma)	Cytomegalovirus
5. AIDS	Papilloma virus
6. Hepatitis	Human immunodeficiency virus
Parasitic STD	
Trichomoniasis	Hepatitis B virus
Fungal STD	
Vulvovaginal candidiasis	<i>Trichomonas vaginalis</i>
	<i>Candida albicans</i>

■ Describe the laboratory diagnosis of STDs.

Collection of Specimens

- Specimens collected are:
 - Urethral discharge
 - Vaginal discharge
 - Cervical specimens
 - Fluid or scrapings from ulcer/vesicle
 - Swab from other lesions
- Specimen is collected with the help cotton or rayon tipped swab. Cotton swab treated with charcoal to absorb toxic material is preferred

Transport of Specimens

The causative organisms of STDs are delicate in nature and do not remain viable outside the body for longer time. Hence, immediate transport and inoculation is necessary. In case of delay, the specimen should be transported to the laboratory in modified Stuart's medium or Amies charcoal transport media held at room temperature.

Microscopic Examination

Different microscopic methods are used for direct demonstration of pathogens in specimen. These include:

- **Gram stain:** This method is used for studying bacterial STDs, particularly valuable for demonstration of gonococci and also for *Candida albicans*
- **Wet mount:** It is the most useful method for rapid detection of *T. vaginalis* in vaginal and urethral discharge and also for detection of *C. albicans*
- **Dark ground microscopy:** This technique is most useful in rapid detection of *T. pallidum* in syphilis
- **Giemsa stain:** This stain can be used for demonstrating inclusion bodies in viral and chlamydial STDs
- **Direct immunofluorescence test:** The test can detect antigens in specimens in viral and chlamydial infections
- **Electron microscopy:** It is valuable in the diagnosis of viral and chlamydial STDs

Culture

To confirm the identity of the causal agent, it is isolated in pure culture. Different culture methods are used for different organisms as follows:

- **Bacterial STDs:** In bacterial infections, specimen is inoculated on bacteriological media. A variety of selective media are used in addition to routine media according to suspected organism based on clinical diagnosis. After appropriate incubation period, the media are observed for growth, which is identified by using standard biochemical and antigenic tests
- **Viral STDs:** In viral STDs, specimen is inoculated in chick embryo and tissue culture. Virus growth after appropriate incubation period is detected by using standard techniques and identified using appropriate techniques
- **Fungal STDs:** For isolation of *C. albicans*, Sabouraud's dextrose agar is used
- **Parasitic STD:** In parasitic STD, cultures are generally not done. Diagnosis is made on the basis of microscopic findings

Ag Detection

Antigens of organisms involved in STDs can be detected by using various tests. These include:

- ELISA
- Latex agglutination test
- Membrane fixed solid phase immunoassay
- Coagglutination test

Ab Detection

Detection of antibody is very important particularly in those STDs in which culture is difficult or not possible. The tests included are, as follows:

- VDRL, *Treponema pallidum* immobilization test, Rapid plasma reagin test, Fluorescent treponemal Ab absorption test and *Treponema pallidum* haemagglutination test are used for Syphilis
- ELISA, Western blot assay, Radioimmunoassay, Immunodiffusion test, Reverse passive haemagglutination test, Counter current immunoelectrophoresis, Agglutination test, etc. can be used for detection of Abs in various bacterial and viral infections

Other Tests

1. Nucleic acid hybridization assay
2. DNA hybridization assay

■ Write a short note on treatment of STDs.

Treatment

Prompt and effective treatment of STDs is desirable to avoid morbidity and long-term problems. Most STDs are curable and virtually all are controllable with appropriate treatment strategy. The following treatment strategy is used:

- Antibiotics for bacterial STDs—ampicillin, penicillin, tetracycline, erythromycin, trimethoprim plus sulphamethoxazole, doxycycline, spectinomycin, etc.
- Antifungal agents for Candidiasis—topical clotrimazole, nystatin, miconazole, etc.
- Metronidazole for trichomoniasis
- In viral infections for
 - Herpes—topical ether, acyclovir (topical or systemic), iodine preparation (topical)
 - Genital warts—25% podophyllin in tincture of benzoin, 5-fluorouracil or liquid nitrogen
 - *Molluscum contagiosum*—preparation containing iodine, phenol or liquid nitrogen (topical)

■ Write a note on nongonococcal urethritis.

Nongonococcal urethritis (NGU) refers to infection of urethra by pathogens other than *Neisseria gonorrhoeae*. NGU is the most common of all the STDs. It constitutes a major health problem. It is a common STD with more than double the incidence of gonorrhoea.

Aetiology

- *Chlamydia trachomatis* (commonest)
- *Ureaplasma urealyticum*
- *Mycoplasma genitalium*
- *Gardnerella vaginalis*
- Herpes simplex virus
- Cytomegalovirus

- *Trichomonas vaginalis*
- *Candida albicans*

Pathogenesis

- Source of infection: Patient or carrier
- Mode of transmission: Through sexual intercourse
- Incubation period: One week to one month or more

Clinical Features

It is characterized by acute mucopurulent urethral discharge, clinically indistinguishable from gonorrhoea where gonococci cannot be demonstrated.

Laboratory Diagnosis

- Specimen: Urethral discharge, cervical discharge, prostatic fluid or urine
- Routine methods of microscopy, culture and serology are used for diagnosis as in STDs

Treatment

- Tetracycline, erythromycin for bacterial infections
- Nystatin and miconazole for *Candida*
- Metronidazole for *T. vaginalis*
- Topical ether, iodine preparations, acyclovir and idoxuridine for HSV

92

Chapter

Hospital Acquired Infections

■ What are hospital infections?

Hospital infections or hospital acquired infections are infections that develop in hospitalized patients, which were not present or in incubation at the time of their admission.

The term “nosocomial infections” is used synonymously for hospital acquired infections. Hospital infections may manifest during the patient’s stay in the hospital or sometimes, after the patient is discharged. Hospital infections also include infections acquired in hospital by hospital staff or visitors to the hospital.

■ Enumerate the most common microorganisms causing hospital infections.

Any pathogen can cause hospital infection, but those that are able to survive in hospital environment for longer periods and develop resistance to antibiotics and disinfectants are particularly important.

Gram-negative bacilli are the major cause, constituting about 60% infections, Gram-positive cocci constitute about 30% infections and 10% infections are caused by fungi, viruses and parasites.

Bacteria

Both aerobes and anaerobes are involved:

Aerobic Bacteria

- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*
- *Escherichia coli*
- *Klebsiella* spp.
- *Proteus* spp.
- *Staph. epidermidis*
- *Streptococcus pyogenes*
- *Haemophilus* spp.
- *Salmonella* spp.
- *Shigella* spp.
- *Mycobacterium* spp.
- *Bacillus subtilis*

Anaerobic Bacteria

- *Bacteroides fragilis*
- *Clostridium* spp.
- Anaerobic cocci

Viruses

- Hepatitis A, B, C and D viruses
- Herpes simplex virus
- Cytomegalovirus
- Influenza virus
- Rotavirus
- HIV

Fungi

- *Candida* spp., particularly *C. albicans*
- *Aspergillus* spp.
- *Mucor* spp.
- *Cryptococcus* spp.
- *Histoplasma* spp.
- *Pneumocystis carinii*

Parasites

- *Entamoeba histolytica*
- *Toxoplasma gondii*

■ What are the sources of hospital infections?

Sources of hospital infections can be endogenous or exogenous.

1. Endogenous

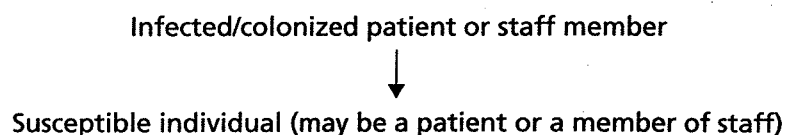
Microorganisms normally present in and on the body of a patient (patients own flora) may act as opportunistic pathogens. These infections are known as endogenous infections, autoinfections or self-infections.

2. Exogenous

Infection from other persons/patients or from inanimate objects in the hospital environment. Hospital infections are mostly exogenous. Exogenous infections include cross-infection and environmental infection described here:

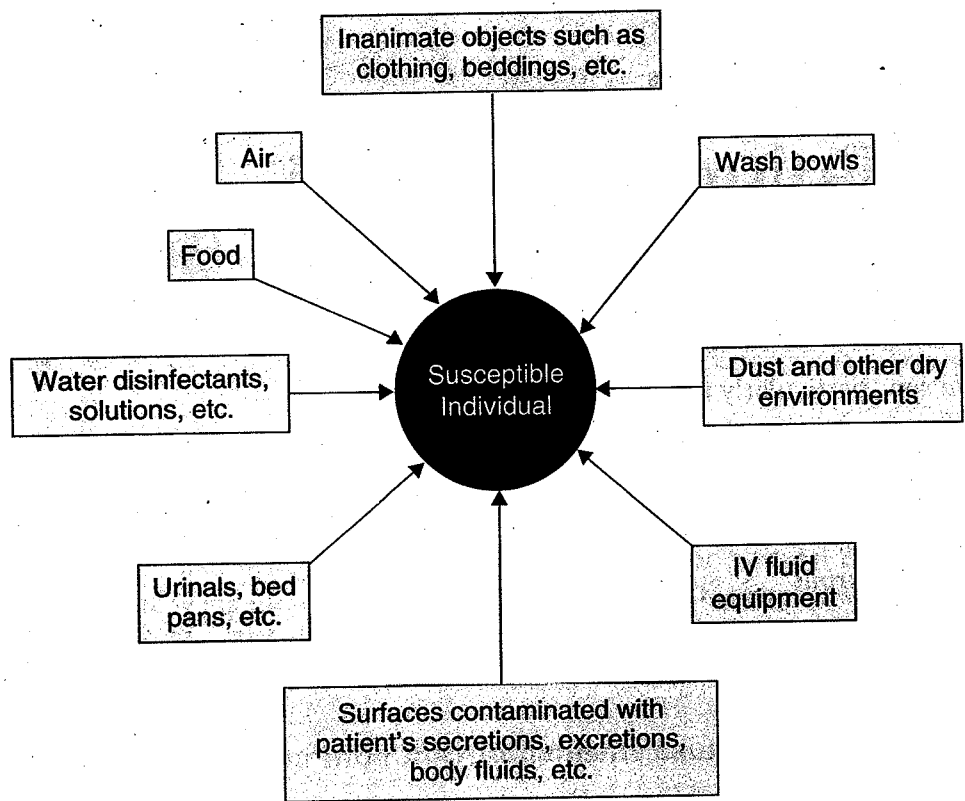
A. Cross-infection

Infection from another patient or hospital staff



B. Environmental infection

Infection from environmental sources such as inanimate objects, air, water, food, etc. (Flowchart 92.1).



Flowchart 92.1 Environmental sources of hospital infection.

■ How are hospital infections transmitted?

Hospital infections spread by the following routes:

- **Contact:** It is the main route of transmission, which occurs by hands or clothings of hospital personnel and even by patient himself or transmitted through contact with inanimate objects
- **Air borne route:** Transmitted by inhalation of droplets, dust from bedding, floors, exudates dispersed from wound, skin, etc.
- **Oral route:** Infection is transmitted by ingestion of contaminated food or water
- **Parenteral route:** Spread of infection is by use of contaminated syringes, needles and other instruments, by administration of contaminated blood, blood products, infusion fluids or tissue
- **Inoculation route:** Infection occurs by inoculation of infected material directly into tissue, e.g. hepatitis B virus infection by transfusion of contaminated blood or inoculation of material containing the virus
- **Iatrogenic transfer:** Infections occur during diagnostic or therapeutic procedures if proper care is not taken. Such infections are called iatrogenic infections

■ Describe the common types of hospital infections.

Following are the common types of hospital infections:

1. Urinary Tract Infections

- Constitute approximately 40% of hospital infections
- Associated with catheterization or instrumentation of urinary tract
- Manifest as pyelonephritis, cystitis, pyelitis or urethritis
- *E. coli*, *Proteus* spp., *P. aeruginosa*, *Klebsiella* spp., coagulase-negative staphylococci, *Serratia* are common organisms

2. Respiratory Tract Infections

- Constitute nearly 15–20% of hospital infections
- Manifest as nosocomial bronchopneumonia
- *Staph. aureus*, *Klebsiella* spp., *P. aeruginosa*, *Enterobacter aerogenes*, *E. coli*, *Proteus* spp., *Acinetobacter* spp. and respiratory viruses are common

3. Wound and Soft Tissue Infections

- Constitute about 18% of hospital infections
- Post-operative wound infections—incidence is high, particularly in elderly patients (above 60 years)
- *Staph. aureus*, *P. aeruginosa*, *E. coli*, *Klebsiella* spp., *Proteus* spp., enterococci and other Gram-negative bacteria are commonly involved
- Infections are of the following two types:
 - Surgical
Post-operative wound infections such as intra-abdominal and pelvic abscesses, infections after orthopaedic surgery, gynaec surgery, surgery on urinary tract, heart surgery, etc.
 - Nonsurgical
 - Stitch abscesses
 - Umbilical stumps
 - Ulcers
 - Burns
 - Injection abscess, etc.

4. Gastro-intestinal Infections

- Transmitted by ingestion of contaminated water or food
- Manifest as:
 - Diarrhoea
 - Dysentery
 - Enteric fever
 - Food poisoning
- GIT infections are most commonly caused by *Salmonella* spp., *Shigella sonnei*, viruses, etc.

5. Other Infections

These include:

- Bacteraemia and septicaemia
- Hepatitis
- Tuberculosis
- Influenza, etc.

■ Comment on the laboratory diagnosis of hospital acquired infections.

- Hospital infection may occur sporadically or as outbreaks
- Aetiological diagnosis is made by routine diagnostic methods of smear, culture and identification
- Antibiotic susceptibility testing is very important because of multiple drug (antibiotic) resistance in nosocomial pathogens
- When it occurs as outbreak, identification and elimination of source are important in controlling the infection
- In outbreak situation, to find out the source, sampling of possible sources—various inanimate objects and hospital personnel including patients who are suspected to be colonized

and also water, food, air, blood products, transfusion fluids, disinfectants, antiseptics, etc., are required

- Detection of carriers among staff and patients is also required
- Typing of isolates from patients and hospital environment is done to find out the source of infection. Various typing methods used are:
 - Phage typing
 - Bacteriocin typing
 - Antibigram or resistogram
 - Biotyping
 - Serotyping
 - Plasmid profile

■ Suggest the methods for prevention and control of hospital-acquired infections.

Prevention

Transmission of hospital acquired infections can be prevented by adopting following practices:

- Education of medical and nursing staff regarding the basic concepts of infection control
- Universal safety procedures to control transmission route
- Aseptic and antiseptic procedures during surgery and after surgery
- Early recognition and isolation of infected cases
- Avoiding overcrowding of patients in a ward
- Detection and treatment of carriers among the patients and staff
- Asepsis in theatre, hygiene in wards and kitchens and special attention to the hospital waste management
- Proper sterilization and disinfection of the inanimate objects and use of disinfectants in the hospital environment for controlling the source or reservoir of infection
- Provision of sterile instruments, dressings, surgical drapes, etc., and use of disposable items such as syringes, catheters, tubing, etc.
- Bacterial interference—colonization of newborn with commensal microorganisms to prevent the colonization of carriage sites by more virulent or antibiotic resistant strains
- Prophylactic antibiotic therapy in specific situations for short period, e.g. before surgical technique
- Limiting the use of antibiotics and a rational use of antibiotics, as per antibiotic policy
- Vaccination to protect the susceptible host

Control

Each hospital should have an effective hospital infection control team (HICT) consisting of microbiologist, medical and nursing staff, hospital administrator and clinician.

Functions of HICT

- Organize surveillance programme for assessing various aspects of infections, such as
 - Investigation of infection
 - Extent of infection
 - Site of occurrence
 - Investigation of possible source
 - Microbial cause
 - Control of outbreak

- Conduct training courses for staff to educate them in the basic concept of infection control
- Prepare training manuals for different categories
- Set and follow guidelines for admission, nursing and treatment
- Implement surveillance systems on sterilization and disinfection practices
- Monitor hygiene practices
- Conduct auditing for evaluating the effectiveness of the various programmes undertaken by HICT committee

93

Chapter

Zoonoses

■ Define zoonoses. Mention the occupational groups at risk.

- Zoonoses are diseases primarily of animals, which can be transmitted to man
- Zoonotic diseases are most commonly acquired by agricultural workers, industrial workers, fish merchants, hunters, slaughtermen (butcher), veterinary surgeons and other animal handlers. The general public is also at risk because of consumption of food of animal origin such as milk, meat, eggs and other products

■ Mention the sources and routes of zoonosis.

The various sources of infection and the mechanism of transmission of infection are presented in Table 93.1.

Table 93.1 Sources and routes of zoonosis

Source	Route
Infected animals	Contact
Contaminated pastures, straw, dust, soil	Inhalation, contact
Milk	Ingestion
Meat	Ingestion
Hides, bones, other animal products	Contact, inhalation

■ How are zoonotic diseases transmitted?

Transmission of zoonotic diseases occurs by the following routes:

- **Animal bite or licks**—introduce microorganisms present in their saliva and on teeth, e.g. inoculation of Rabies virus in tissue by dog bite
- **Eggs**—laid by infected hen act as a disease vector. Poultry are frequently carriers of *Salmonella* spp.
- **Direct contact with animals**—e.g. brucellosis in farmers or veterinary personnel by handling genital tract of a cow
- **Ingestion of food and water contaminated by animals**—food and water may be contaminated with faeces and urine
- **Blood and live tissues**—e.g. animal handlers, zoo and laboratory workers may get infection by direct handling or by inhaling aerosols from infected blood or tissues of animals, e.g. yellow fever
- **Food and water**—may be contaminated with other animal products or there may be a pre-existing infection (in meat), e.g. fish infected with organisms acts as a source of infection. Also the consumption of animal products such as milk, meat, eggs or dairy products lead to infection, e.g. unpasteurized milk is the potential source of a number of zoonotic diseases

- **Insects**—e.g. blood sucking insects may transmit pathogens from animal to human beings
- **Inhalation** of air contaminated (infected aerosols) by animals—air contamination may occur due to shedding of organisms from skin of animals by air current, from animal excreta or from other animal products, e.g. pulmonary anthrax by inhalation of spores in wool.

■ Classify zoonoses on the basis of the lifecycle of the infecting organism.

On the basis of the lifecycle of the infecting organism, zoonosis is of the following types:

1. Direct zoonoses

The infecting organism requires a single vertebrate host, e.g. Rabies.

2. Cyclozoonoses

The infecting organism requires more than one vertebrate host to complete its lifecycle, e.g. *Echinococcus granulosus*.

3. Metazoonoses

The infecting organism requires a vertebrate host and an invertebrate vector for its biological transmission, e.g. malarial parasite, *Leishmania donovani*.

4. Saprozoonoses

The infecting organism requires a vertebrate host and nonanimal reservoir, e.g. *Histoplasma capsulatum*.

■ Classify zoonoses on the basis of aetiological agents. Name these agents, the diseases caused by them and the animals in which are present.

Based on aetiological agents, zoonoses can be classified into the following types:

- **Bacterial zoonoses:** Zoonoses caused by bacteria
- **Viral zoonoses:** Zoonoses caused by viruses
- **Parasitic zoonoses:** Zoonoses caused by parasites
- **Fungal zoonoses:** Zoonoses caused by fungi

Specific agents causing zoonoses, names of the diseases caused by them and the animals which they affect are listed in Table 93.2.

■ Write a note on the laboratory diagnosis of zoonoses.

Diagnosis is important for surveillance of zoonoses. It is achieved, in humans and animals, based on

- Isolation of causative agent
- Serological surveillance
- Specimen inspection or autopsy

For the laboratory diagnosis routine methods are used in both, humans and animals. These include:

Collection of Specimens

Specimens are collected according to the site of lesions. In human beings, various specimens are collected according to the site of infection. In case of infected animals, following specimens are collected:

- Ear specimen, e.g. anthrax
- Swabs soaked in blood, e.g. anthrax

Table 93.2 Zoonotic diseases and their casual agents

Causal agent	Disease	Animal test
Bacterial zoonoses		
1. <i>Brucella abortus</i>	Brucellosis	Cattle
2. <i>B. suis</i>	Brucellosis	Pigs
3. <i>B. melitensis</i>	Brucellosis	Goats, sheep
4. <i>Bacillus anthracis</i>	Anthrax	Cattle
5. <i>Francisella tularensis</i>	Tularemia	Squirrels, rodents
6. <i>Leptospira interrogans</i>	Leptospirosis	Swine, dogs, cattle, rats
7. <i>Salmonella</i> spp.	Salmonellosis (Food poisoning)	Cattle, poultry, pigs
8. <i>Yersinia pestis</i>	Plague	Rats
9. <i>Mycobacterium bovis</i>	Tuberculosis	Cattle
10. <i>Yersinia pseudotuberculosis</i>	Mesenteric Adenitis	Various animals
11. <i>Chlamydia psittaci</i>	Psittacosis	Parrots and other birds
12. <i>Rickettsia</i> species	Rickettsial fever	Birds, rodents, poultry, cattle, etc.
13. <i>Pseudomonas mallei</i>	Glanders	Horses
Viral zoonoses		
1. Rabies virus	Rabies	Dog, cat, fox
2. Japanese encephalitis virus	Japanese encephalitis	Pigs and cattle
3. Kyasanur forest virus	Kyasanur forest disease	Forest birds and monkeys
4. Yellow fever virus	Yellow fever	Monkeys
5. Influenza virus	Influenza	Swine, horse, birds
Parasitic zoonoses		
1. <i>Echinococcus granulosus</i>	Hydatid disease	Sheep, cattle, dog
2. <i>Toxoplasma gondii</i>	Toxoplasmosis	Dog, cat, hen
3. <i>Taenia saginata</i>	Taeniasis	Cattle
4. <i>T. solium</i>	Taeniasis	Pig
5. <i>Leishmania donovani</i>	Leishmaniasis	Dog
6. <i>Plasmodium</i> spp.	Malaria	Various animals and birds
Fungal zoonoses		
1. <i>Trichophyton</i> spp.	Dermatophytoses	Dog and cat
2. <i>Microsporum</i> spp.	Dermatophytoses	Dog and cat
3. <i>Histoplasma capsulatum</i>	Histoplasmosis	Birds

- Carcass of animal, e.g. rabies
- Severed head, e.g. rabies
- Pooled milk sample, e.g. brucellosis
- Pieces of kidneys, e.g. leptospirosis
- Autopsy specimens, in various diseases

Transport of Specimen

Specimen is transported immediately by taking all aseptic precautions.

Microscopic Examination

The different microscopic methods used are:

- **Gram staining**—for bacterial and fungal zoonoses
- **Electron microscopy**—for virus, chlamydia, rickettsia
- **Saline and Iodine preparations**—for parasitic zoonoses
- **KOH mount**—for fungal zoonoses
- **Demonstration of inclusion bodies**—in viral and chlamydial zoonoses
- **Direct immunofluorescence stain**—for demonstration of antigen in various zoonotic diseases

Culture

- Routine culture on bacteriological (both selective and nonselective) media for bacteria
- Tissue culture and chick embryo for virus
- Sabouraud's dextrose agar and other media for fungal culture

The media are observed for growth after appropriate incubation period and identified using standard methods.

Serological Methods

Serological tests are particularly important in those zoonotic diseases in which culture is difficult or impracticable. The various serological tests used are as follows:

- Agglutination test
- Precipitation test
- Complement fixation test
- Radioimmunoassay
- Enzyme-linked immunosorbent assay
- Indirect immunofluorescence test
- Haemagglutination test
- Neutralization test, etc.

Skin Test

Allergic skin test is also helpful in some zoonotic diseases.

Treatment

- Antibiotics for bacterial zoonoses
- Antifungal agents for fungal zoonoses
- Antiparasitic agents for parasitic zoonoses
- No specific therapy for viral zoonoses

94

Chapter

Bacteriology of Water, Milk and Air

■ Discuss the role of water in transmitting infection.

- Water is the most important vehicle for a variety of pathogens causing intestinal or systemic infections, particularly in developing countries, because of its contamination with sewage or other excreted material
- Water is said to be contaminated (polluted) when it contains infective and parasitic agents, poisonous chemical substances, industrial or other wastes or sewage
- The hazards of water pollution are of two types:
 1. **Biological hazards**—hazards due to infective agents, which may lead to water-borne diseases
 2. **Chemical hazards**—hazards due to chemical poisonous substances
- Faecal contamination of water supplies may lead to water borne-diseases. The objective of microbiological study of water supplies is to detect pollution of water by pathogenic microorganisms
- Demonstration of possible pathogen in water is ideal but practically it is difficult as their number is very less and outnumbered by nonpathogenic microorganisms

Hence, faecal pollution of water is demonstrated by detecting indicator organisms of human/animal faecal pollution, i.e. intestinal microorganisms.

■ What is meant by safe drinking water?

Safe water (potable water) is one that cannot harm the consumer, even when ingested over a prolonged period.

Drinking water should not only be safe but also pleasant to drink, i.e. clear, colourless and devoid of unpleasant taste, smell or appearance.

■ Write a short note on bacterial flora of water.

The bacteria found in water can be divided into three groups:

1. Natural water bacteria

These are the bacteria naturally present in water. They are commonly found in water and are not considered as pollutants, e. g.

- *Micrococcus*
- *Serratia*
- *Alkaligenes*
- *Pseudomonas*
- *Flavobacterium*
- *Acinetobacter*

2. Soil bacteria

These are the bacteria washed into water with soil during rainy season. These are not normal inhabitants, e. g.

- *Bacillus subtilis*
- *B. megaterium*
- *Enterobacter cloacae*
- *B. mycoides*
- *E. aerogenes*

3. Sewage bacteria

These are the normal inhabitants of the intestine of humans and animals. Bacteria decomposing organic matter may sometimes be present in water because of contamination of water bodies with sewage, e. g.

1. Intestinal bacteria
 - *Escherichia coli*
 - *Streptococcus faecalis*
 - *Salmonella typhi*
 - *Vibrio cholerae*
2. Sewage proper bacteria (Bacteria decomposing organic matter)
 - *Proteus vulgaris*
 - *Clostridium sporogenes*
 - *Nocardia* spp.

■ Name the disease-causing pathogens present in polluted water.

Contamination of water with sewage may introduce a variety of pathogens. Polluted water can transmit the following pathogens:

1. Bacteria

- *V. cholerae*
- *S. typhi*
- *Shigella* spp.
- *Yersinia enterocolitica*
- *E. coli*
- *S. paratyphi* A, B and C

2. Viruses

- Hepatitis A virus
- Poliovirus
- Hepatitis E virus
- Rotavirus

3. Protozoa

- *E. histolytica*
- *B. coli*
- *Isospora*
- *Giardia lamblia*
- *Cryptosporidium*

4. Helminths

- Round worm
- Whip worm
- Guinea worm
- Thread worm
- Dog tapeworm
- Fish tapeworm

■ Comment on indicator bacteria.

- An indicator organism is one which is abundantly present in faeces and scanty in other sources
- Indicator bacteria are easy to isolate and identify
- They are unable to grow, but can survive in water for longer periods than other pathogens
- Their resistance to disinfectants such as chlorine is more than other pathogens
- The organisms most commonly used as indicators of pollution are:
 - *E. coli* in particular and coliform as a whole
 - *Streptococcus faecalis* (faecal streptococci)
 - *Clostridium perfringens*
 - Anaerobes such as bifidobacteria and bacteroides—are more abundant than coliform but are difficult to detect and enumerate, hence not used
 - Faecal streptococci are regularly present but their number is less than *E. coli*. Their presence along with coliforms in absence of *E. coli* confirms faecal pollution
 - *Cl. perfringens*—present in small number—their spores survive longer as they are resistant to chlorination. Their presence indicates faecal contamination and absence of coliforms suggests that it occurred quite ago

■ How is bacteriological examination of water carried out?

The steps involved in bacteriological examination of water are as follows:

Collection of Water

- Water should be collected regularly and frequently to find out seasonal variations in the quality of water
- Sample should be collected, stored and dispatched in suitable sterilized bottles
- Collection of sample should be in sterilized container with 0.1 ml of sodium thiosulphate to neutralize the bactericidal effects of chlorine or chloramines
- Volume of water should be large (200–300 ml) enough to permit accurate analysis
- Great care should be taken to avoid contamination during sampling and it should be dispatched as soon as possible to prevent significant change in the composition of microflora of water

Type of Water to be Sampled

Sample from tap

- The dirt from the tap is removed with the help of clean cloth
- Tap water is then allowed to flow fast for 3–5 minutes
- Tap is closed and sterilized for 1–2 minute with flame
- Again tap water is allowed to flow for 1 or 2 minutes at minimum rate
- Container is opened and required quantity of water from tap is allowed to flow in by holding the bottle under the water jet
- A small airspace is left to facilitate shaking at the time of inoculation

- The container is stoppered and labelled properly with details of the source, time and date of collection and transported to laboratory as soon as possible (at least within 6 hours in a cool container and protected from light)

Sample from reservoir

- For collection of water from streams, rivers, tanks and lakes, the stopper is opened and the bottle is filled by holding it at the lower part, submerging it to a depth of 20 cm, with the mouth facing slightly upwards
- If there is a current, the bottle should face the water current

Sample from a well

- A stone of suitable size is attached to the sampling bottle with a piece of thread
- A clean thread or string of suitable length is tied with the bottle and lowered to the required depth
- When the bottle is filled, it is pulled out and stoppered properly

Transport

- After the bottle is closed, it is wrapped in a Kraft paper
- Labelled properly—source, time and date
- Transported immediately to laboratory—within 1 hour and in no case testing should be delayed beyond 6 hours
- When delay is expected, sample should be kept in ice box and protected from light

Methods of Analysis

Multiple Tube Method

- In this method, the most probable number (MPN) of coliform bacilli present in the water sample can be determined statistically
- **Media:** Double strength and single strength MacConkey's broth with inverted Durham's tube for indication of gas production are used
- **Procedure:** Measured quantities of water sample and medium are mixed in following composition:
 - 50 ml of water is added to 50 ml of double strength medium
 - 10 ml of water is added to each of the 5 tubes of 10 ml double strength medium
 - 1 ml of water is added to each of the 5 tubes of 5 ml single strength medium
- **Incubation:** The set of tubes and bottles are incubated at 37°C for 18–24 hours and examined. It is reincubated further for 24 hours and examined
- **Results**
 - Tube showing formation of acid (change in colour) and gas bubbles (tapped in Durham's tube) is considered as positive
 - An estimate of coliform count per 100 ml is made from the tubes/bottles showing positive results using the probability tables of McCrady
 - The presumptive coliform count is reported as follows:

Presumptive coliform count	Class
0	Excellent
1–3	Satisfactory
4–10	Suspicious
More than 10	Unsatisfactory

- **Demonstration of type of bacteria:** The type of coliform bacteria present in the water sample is determined by the following tests:
 - a. **Eijkman test** (differential coliform test or confirmed *E. coli* count)
 - This test is done to confirm that the coliform bacilli detected in the presumptive test are *E. coli*, as some spore forming bacteria give false positive reaction in the presumptive coliform test
 - After the presumptive test, subcultures are made from all tubes showing acid and gas to fresh tubes of single strength MacConkey's medium
 - These tubes are incubated at 44°C in water bath and examined after 24 hours
 - Tubes showing acid and gas are considered to contain *E. coli* and final confirmation is made by plating on solid media and by testing indole production
 - b. **Detection of faecal streptococci and *Clostridium perfringens***
 - In doubtful cases, for detection of faecal pollution these two organisms are considered as indicators
 - Their presence in water shows faecal contamination of water in doubtful cases
 - For detection of faecal streptococci, subcultures from presumptive positive tubes in coliform test are made into tubes containing 5 ml of glucose azide broth and incubated at 45°C for 18 hours
 - Production of acid in the medium indicates the presence of *Streptococcus faecalis*
 - Further confirmation can be done by plating onto MacConkey's agar
 - For detection of *Cl. perfringens*, the water sample is inoculated into litmus milk medium and incubated anaerobically at 37°C for 5 days
 - Typical stormy fermentation with acidity confirms the presence of *Cl. perfringens*

Membrane Filter Method

- A measured volume of water is filtered through a membrane filter that retains bacteria on its surface
- The membrane is then placed on a suitable selective medium and incubated allowing the bacteria to grow and form colonies
- The number of colonies is counted and the bacteriological content of the water is calculated
- **Advantage:** Results are available in 20 hours
- **Disadvantage:** The method is not suitable for testing turbid water

Examination of Specific Pathogens

- Specific pathogens may also be isolated from water when indicated, e.g. during an outbreak or for evaluation of a new source
- Pathogens like, *Salmonella* spp., *Shigella* spp., vibrios, *E. coli*, etc. may be isolated by using defined standard procedure for individual organism
- In general, the following steps are followed for their isolation and identification:
 - Concentration of organism in the sample
 - Inoculation into enrichment broth
 - Subculture on selective media
 - Biotyping and serotyping for identification and confirmation
- Example: For isolation of *S. typhi*, equal volume of water is added to the double strength selenite broth and incubated and subcultured on selective medium. Isolated organisms are identified by biochemical tests and serotyping
- Pathogenic organisms may also be isolated by membrane filter method

■ **Mention the types of bacteria found in milk.**

- Milk supports the growth of a variety of bacteria including pathogenic one
- The different types of bacteria present in milk are as follows:

1. **Acid-forming bacteria**, such as

- *Streptococcus lactis*
- *Str. faecalis*
- Lactobacilli

These ferment lactose, forming lactic acid, and lead to the formation of curd.

2. **Alkali-forming bacteria**, such as

- *Alkaligenes* spp.
- *Achromobacter*
- Aerobic spore-forming bacilli

These render the milk alkaline.

3. **Gas-forming bacteria**, such as

- Coliform bacteria
- *Cl. perfringens*
- *Cl. butyricum*

These produce acid and gas.

4. **Proteolytic bacteria**, such as

- *Bacillus subtilis*
- *B. cereus*
- *Proteus vulgaris*
- Staphylococci
- Micrococci

These bacteria are responsible for proteolytic activity.

5. **Inert bacteria**, such as

- *Achromobacter*
- Pathogenic bacteria
- Cocci

They do not produce any visible change.

■ **Enumerate the milk borne diseases and infections.**

Diseases and infections transmitted through milk are as follows:

1. **Infections of animals transmitted to man**

These include:

- Tuberculosis
- Brucellosis
- Streptococcal and staphylococcal infections
- Salmonellosis
- Q fever
- Anthrax
- Leptospirosis
- Cowpox and milker's nodes
- Foot and mouth disease
- Tickborne viral encephalitis

2. Infection due to ingestion of milk contaminated with excreta of small mammals

- *Streptobacillus moniliformis*
- *Campylobacter jejuni*
- *Yersinia enterocolitica*

3. Infections primarily of man transmitted through milk

- *S. typhi*
- Paratyphoid bacilli
- *Cholera vibrio*
- *Shigella*
- *E. coli*
- Streptococcal and staphylococcal infections
- Tubercle bacilli
- Hepatitis virus
- Diphtheria bacilli

■ Describe the tests used in bacteriological examination of milk.

The tests used for examining bacteria present in milk are as follows:

1. Viable count

- It is detected by plate counts with serial dilutions of the milk samples
- Raw milk is diluted with sterile ringer saline as 1:10, 1:100 and 1:1000. 1 ml of appropriate dilution is mixed with 10 ml of medium and poured in plates
- Plates are incubated at 30°–31°C for 72 hours
- Number of colonies are counted and multiplied by dilution factor and number of viable bacteria per millilitre of milk is determined
- Raw milk contains 500 to several million bacteria per millilitre

2. Coliform count

- For this, different dilutions of milk are inoculated into MacConkey's liquid medium
- The production of acid and gas is noted after incubation—positive test indicates presence of coliform bacilli (in two tubes out of three)
- All coliforms are killed by pasteurization
- The presence of coliforms in pasteurized milk indicates improper pasteurization or post-pasteurization contamination

3. Methylene blue reduction test

- An indirect method for detection of microorganisms in milk
- An economical substitute for viable count
- **Procedure:** 1 ml of methylene blue is mixed with 10 ml of milk in a test tube and incubated in dark at 37°C
- **Result**
 - If bacteria are present, the milk is decolourized after reduction of methylene blue by bacteria making the milk colourless—positive test
 - No change in colour—negative test
 - Milk is considered satisfactory if it fails to decolourize methylene blue in 30 minutes

4. Resazurin test

- It is also a dye reduction test similar to methylene blue dye test
- Reduction of resazurin by bacteria passes through a series of colour changes—blue to pink to colourless, which depends upon degree of contamination

5. Phosphatase test

- Phosphatase is an enzyme normally present in milk, which gets inactivated by pasteurization of milk
- Its presence in milk after pasteurization indicates improper/incomplete pasteurization
- Its presence is detected by adding disodium phenyl phosphate to 1 ml of milk in a test tube and incubating in a water bath at 37°C for 2 hours
- Development of yellow colour indicates presence of phosphatase

6. Turbidity test

- This test is used to check sterilization of milk
- When milk is sterilized properly by heating at 100°C for 5 minutes, all coagulable proteins are precipitated and when ammonium sulphate is added to this milk, no turbidity results because of denaturation of proteins. This indicates that milk has been heated at 100°C for 5 minutes

■ Describe the tests used for examining specific pathogens present in milk.

Examination of milk for specific pathogens can be done by the following methods:

1. Test for tubercle bacilli

- The milk is centrifuged at 3000 rpm for 30 minutes and sediment is inoculated into two guinea pigs and two L-J media
- L-J media observed for growth and guinea pigs are observed for a period of 3 months for tuberculosis
- Confirmation is done by using biochemical tests

2. Tests for *Brucella bacilli*

- Isolation of brucellae is attempted by
 - a. Inoculating cream from the milk sample on serum glucose agar
 - b. Injecting centrifuged deposit of the milk sample intramuscularly in guinea pigs
- The guinea pig can be sacrificed after 6 weeks and the spleen used for culture of brucella and serum used for demonstration of Abs by milk ring test
- **Milk ring test** is the highly sensitive test for demonstration of brucella Abs in the milk of infected cows. The test is used for diagnosis of brucellosis in animals. The positive milk ring test is confirmed by Whey's agglutination test
- In case of suspected food poisoning, the sediment of centrifuged milk should also be examined for:
 - *Staph. aureus*
 - *Salmonella* spp.
 - *Campylobacter* spp.
 - *Y. enterocolitica*

■ Discuss bacteriology of air.

- Air always contains foreign substances including microorganisms and it is never pure
- The microbes are present in air in all places at all times

- The level of bacterial contamination is expressed as the number of bacteria carrying particles per m^3 or per ft^3
- Since an adult male inhales about 15 m^3 and a baby about 1 m^3 of air in a day, the bacterial content of air is important, particularly when the air contains pathogenic microorganisms
- Possibility of a person to acquire infection will be more if he is exposed to high concentration of airborne pathogens
- Humans contribute significantly in spreading microbes in the environment, e.g. during talking, coughing and sneezing
- The bacterial content of air depends on
 - Increased density of human and animal population
 - Nature of soil
 - Amount of vegetation
 - Atmospheric conditions such as humidity, temperature, wind, rainfall and sunlight
- The bacterial content of air also depends on location. Outdoor air contains usually non-pathogenic organisms, however indoor air contains droplets of organisms disseminated by humans and animals, which may include pathogens.

■ **Give the name of important setups where bacteriological examination of air is required?**

Bacteriological examination of air is required in:

- Surgical operation theatres
- Hospital wards
- Storehouses or premises of food articles or pharmaceutical preparations

■ **Describe the methods for carrying out bacterial examination of air and environmental dust.**

Bacteriological Examination of Air

- Measuring the rate at which bacteria carrying particles settle down, e.g. settle plate method
- Counting the number of bacteria carrying particles contained in a given volume of the air, e.g. slit sampler method

Settle Plate Method (Sedimentation Method)

- Petri plates containing culture media are exposed to air for 30 to 60 minutes
- Nutrient agar or blood agar (for streptococci and staphylococci) or selective media for special pathogens can be used
- Large bacteria carrying dust particles settle onto the medium
- The plates are incubated at 37°C for 24 hours
- The number of colonies is counted and the number of bacteria present in air is calculated
- It is simple but measures only the rate of deposition of large particles from air but not the total number of large and small bacteria carrying particles in air
- Uses: Testing air in surgical operation theatres and hospital wards

Slit Sampler Method

- It determines the number of bacteria in a measured volume of air
- It is better than settle plate method, as it is efficient in detection of very small particles also
- In this, a known volume of air is directed onto a plate of culture medium through a slit of 0.25 mm width. The plate is rotated mechanically in order to ensure uniform distribution of microorganisms all over the plate-containing medium

- One ft³ of air is allowed to pass through the slit and in a similar manner 10 ft³ air is tested
- The plates are incubated and colonies are counted
- The number of colonies gives the number of bacteria present in air

Air Contamination Standards

Bacterial count should not exceed the upper limit of:

- 10/ft³ in operation theatre
- 1/ft³ in operation theatres for neurosurgery and burns
- 50/ft³ in factories, offices and homes

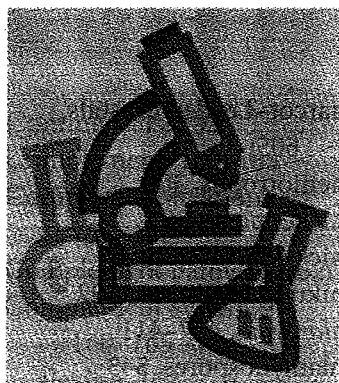
Bacteriological Examination of Environmental Dust

Sweep Plate

- In this, a Petridish containing suitable culture medium is rubbed over surfaces such as clothing, bed cloth, curtains, etc. with the medium facing the surface allowing dust particles to settle on the medium
- Plate is incubated and colonies are counted after incubation
- Method is useful in detecting organisms from various surfaces

Dust Sampling

- Moistened cotton swabs are rubbed on the floor, wall, furniture and other surfaces for dust sampling
- The swabs are then placed in broth and Robertson's cooked meat media and incubated
- After subculturing on plates, the isolates can be identified
- This is routinely used for assessing the level of asepsis in operation theatres, especially for detection of spores of *Cl. tetani* and other clostridia in theatre dust



Index

A

- Ab dependent cell
 - cytotoxicity, 187
- Ab-mediated immune response, 172
- Abnormal immunoglobulins, 141
 - Bence-Jones proteins, 141
 - multiple myeloma, 141
- Abnormal replicative cycles, 429–430
- Acanthamoeba*, 608
- Acid fast stain, 17
- Acidophilic bacteria, 132
- Acinetobacter calcoaceticus*, 418
- Acquired immunity, 13
- Actinomadura madurae*, 562
- Actinomyces* spp., 396
- A. israelii*, 396
- Actinomycosis, 397
- Active immunity, 126–128
 - artificially acquired, 127–128
 - naturally acquired, 127
- Acute glomerulonephritis, 249
- Acute rheumatic fever, 249
- Adansonian classification, 39
- Adenoviruses, 472–474
- Adjuvants, 185
 - Freund's complete adjuvant, 185
 - Freund's incomplete adjuvant, 185
- Agar, 41
- Affinity, 142
- Aflatoxin, 593
- Agar dilution test, 119
- Agar gel diffusion, 146
- Agglutination reaction, 149–152
- AIDS, 199, 527t
- Albert's stain, 265
- Alexander Fleming, 8
- Algid malaria, 624
- Alleles, 89
- Allergy, 200–209
- Allograft, 216
- Alpha-haemolysis, 172t
- Alpha toxin, 280
- Alternate pathway, 163
- Amboceptor, 152
- Amoebiasis, 605
- Anaerobic media, 46
- Anaerobiosis, 138
 - methods of, 50
- Anal swab, 263
- Anaphylactoid reaction, 205
- Anaphylaxis, 201–202
- Ancylostoma duodenale*, 652–654
- Andrade's indicator, 46, 56
- Anthrax, 274
- Anthraxoid bacilli, 276t
- Antibodies, 135
- Antigen, 131–134
 - complete, 131
 - haptens, 131
 - incomplete, 131
 - properties of, 132–133
 - schlepper, 131
- Antigen-antibody reactions, 142–159
- Antigenic determinant, 132
- Antigenic drift, 483
- Antigenic shift, 483
- Antigenic specificity, 133–134
- Antigen presenting cells, 176

- Antimicrobial susceptibility testing, 115–120
- Antisera and antitoxins, 225
- Antony van Leeuwenhoek, 6
- Arboviruses, 496
- Arnold sterilizer, 68
- Arthropod borne diseases, 104
- Arthus reaction, 207
- Asbestos filters, 72
- Ascariasis, 650
- Ascaris lumbricoides*, 649–652
- Ascaris pneumonia*, 650
- Ascending UTI, 696
- Ascoli's thermo precipitation test, 274
- ASO test, 250
- Aspergillosis, 583
- Astroviruses, 534
- Athletes foot, 556
- Atypical mycobacteria, 374–377
- Atypical pneumonia, 686
- Australia Ag, 513
- Autoclave, 68–70, 81
- Autoimmune disease, 211
- Autoimmunity, 210–214
- Atopy, 205
- Autograft, 215
- Autospecificity, 133–134
- Autotrophs, 29–30
- Avidity, 142
- Azidothymidine, 446

B

- Babes-Ernst granules, 265
- Bacille Calmette-Guerin (BCG), 185, 373

- Bacillus anthracis*, 272
bamboo-stick appearance, 272
Bacillus cereus, 276
BACTEC, 331, 371
Bacteraemia, 109, 667-672
Bacterial cell, 19-28
Bacterial genetics, 84-101
Bacterial growth, 31
Bacterial growth curve, 32-33
Bacterial metabolism, 34-35
Bacterial spores, 27-28
Bacterial vaginosis, 417
Bacteriocin typing, 331
Bacteriological index, 378-379
Bacteriology of air, 721
Bacteriology of milk, 719
Bacteriology of water, 714
Bacteriophage typing, 454
Bacteriophages, 451-455
Bacteriuria, 695
Bacteroides spp., 291
Bulantidium coli, 629-630
Bartonellae, 405-406
Basal medium, 42
B cells, 173
Beef tapeworm, 633-636
Bergey's manual, 36-37
Beta-haemolysis, 245t
Bile solubility, 253
Bilharziasis, 642
Binary fission, 32f
Biological false positive reactions, 387-388
Biphasic culture, 313
Bisected pearls, 347
Black death, 343
Black water fever, 624
Blastomyces dermatitidis, 577
Blastomycosis, 577
Blood culture, 671
B lymphocytes, 173
Bordetella, 347-351
 B. bronchiseptica, 347
 B. parapertussis, 347
 B. pertussis, 347
Borrelia spp., 292
 B. burgdorferi, 390
 B. duttoni, 390
 B. recurrentis, 390
 B. vincenti, 390
Botulism, 289
 food-borne botulism, 289
 infant botulism, 289
 wound botulism, 289
Bronchopneumonia, 686
Broth dilution method, 242
Brucella spp., 352
Brucellosis, 353
Brugia malayi, 663-664
Bubonic plague, 343
Burkholderia, 333
Burkitt's lymphoma, 470
Bursa of Fabricius, 168
- C**
Calibrated loop method, 698
Calymmatobacterium granulomatis, 417
Campylobacter, 418
Candida albicans, 571f
Candidiasis, 568
Candle filters, 72-73
Candle jar, 50
Capnophilic bacteria, 50
Capsid, 422
Capsomeres, 422
Capsule, 24, 108
Cardiolipin Ag, 385
Carpet culture, 48
Carriers, 103-104
 types of, 103-104
Casoni's test, 637
Castaneda's method, 354, 671
Catalase test, 60
Cat scratch disease, 406
Cell cultures, 433-434
Cell lines, 432
Cell-mediated immune response, 196
Cell-mediated immunity, 187
Cell membrane, 22-23
Cell wall, 20-22
Cellular factors, 125-126
Cellular immunodeficiencies, 196-197
Central (primary) lymphoid organs, 167-168
Cerebral malaria, 624
Cestodes, 633-640
Chancre, 385
Chancroid, 362
Chandipura virus, 500
Charcot-Leyden crystals, 605t
Chemotrophs, 30
Chickenpox, 466
Chick Martin test, 78
Chikungunya virus, 592
Chlamydia, 407-411
Chlamydispores, 543
Cholera red reaction, 335
Chromoblastomycosis, 567
Chromomycosis, 549
Chronic mucocutaneous candidiasis, 197
Cigar bundle appearance, 378
Ciliata, 599
Citrate utilization test, 58-59
Citrobacter, 304
Class I antigens, 177
Class II antigens, 177-178
Class III antigens, 178
Classical complement pathway, 162f
Classification of viruses, 436t
Clean-catch midstream urine collection, 697
Clonal deletion, 192
Cloning vectors, 75
Clostridia, 278
 antibiotic-associated pseudomembranous colitis, 290
Clostridium perfringens, 278
Clostridium tetani, 283
Clostridium welchii, 279f
Clot culture, 319
Clue cells, 413
Co-agglutination test, 151f
Coagulase, 239
 bound coagulase, 239
 free coagulase, 239
Coagulase test, 61-63
Coccidioides immitis, 578
Coccidioidomycosis, 580
Codon, 86
Cold agglutination test, 415
Col-factor, 95
Combined immunodeficiencies, 197-198
 ataxia telangiectasia, 197
 Nezelof syndrome, 197
 severe combined, 198
 Wiskott-Aldrich syndrome, 197

- Commensals, 102
 Complement, 160–165
 Complement fixation test, 152–153
 Complement pathway, 162f
 Complement system, 160–165
 alexine, 160
 Complex media, 42
 Complex symmetry, 424
 Coombs and Gel classification, 201
 Coomb's antiglobulin test, 150
 Condylomatas, 386
 Congenital rubella, 532
 Congenital syphilis, 386
 Congenital transfer, 106
 Conglutination, 165
 Conjugation, 93–94
 Contact dermatitis, 209
 Continuous cell lines, 433–434
 Continuous culture, 34
 Corn-meal agar, 571
 Corona viruses, 534
Corynebacterium diphtheriae, 236
 Chinese letter pattern, 265
 cuneiform pattern, 265
Corynebacterium pseudotuberculosis, 265–271
 Counter current immunoelectrophoresis, 147–148
 Cowpox, 458
Coxiella burnetii, 404–405
 Cocksackie virus, 478
 Craigie's tube technique, 315
 C-reactive protein, 253–254
 Creeping eruptions, 664
 Creutzfeldt–Jakob disease, 536
 Croup, 491
 Cryoglobulinaemia, 141
 Cryptococcosis, 572
Cryptococcus neoformans, 683
 Cryptosporidiosis, 630
Cryptosporidium parvum, 630–631
 Cyclophyllidean cestodes, 600
 Cysticercosis, 633–634t
 Cultivation of viruses, 431
 Culture media, 40–46
 Culture methods, 47–53
 Cutaneous mycoses, 552
 Cutaneous or local anaphylaxis, 204–205
 Cyclosporine, 218
 Cytocidal or cytolytic test, 154
 Cytokines, 188
 Cytomegalovirus, 467
 Cytopathic effects, 434
 Cytoplasm, 23
 Cytoplasmic membrane, 22
 Cytotoxic hypersensitivity, 206f
 Cytotoxic T-cells, 173, 191
D
 Dane particle, 512
 Darting motility, 334
 Decline phase, 32–33
 Deep mycoses, 561–582
 Deficiency of innate immunity, 194
 Definitive host, 633–634t
 Delayed hypersensitivity, 208
 Delhi boil, 617
 Delta agent, 517
 Dendritic cells, 176
 Dengue fever virus, 500–501
 Dengue haemorrhagic fever, 500
 Dermatophytes, 554–555
 Dermatophytoses, 556–557
 Detection of CMI, 191
 Diarrhoea, 689–694
 Dienes' Phenomenon, 309
 Dienes stain, 413
 Differential media, 45
 Di George's syndrome, 197
 Dimorphic fungi, 545
 Diphtheria, 267–268
 Diploid cell strains, 433
 Disc diffusion method, 115–116
 Disinfection, 73
 acids and alkalis, 76
 alcohols, 75
 alkylating agents, 76–77
 ethylene oxide, 77
 formaldehyde, 76
 glutaraldehyde, 77
 beta propiolactone, 77
 dyes, 76
 Disinfection (*Continued*)
 heavy metals, 76
 oxidizing agents, 75–76
 halogens, 75
 chlorine, 75
 iodine, 75
 hydrogen peroxide, 75
 potassium permanganate, 76
 phenols, 74
 surface active agents, 77–78
 Disorders of complement components, 195
 Disorders of phagocytosis, 194
 Chediak–Higashi disease, 195
 chronic granulomatous disease, 194–195
 lazy leucocyte syndrome, 195
 leucocyte glucose 6 phosphate dehydrogenase deficiency, 195
 myeloperoxidase deficiency, 195
 tuftsin deficiency, 195
 Disposal of waste, 81
 DNA probes, 99–100
 DNA recombinant technology, 97–98
 DNA structure, 85f
 DNA viruses, 422t
 Dog tapeworm, 716
 Donovan bodies, 417
Donovania granulomatis, 417
 Donovanosis, 417
 DPT, 287
 Dracunculosis, 665–666
 Drug resistance, 95
 mutational, 95–96
 transferable, 96
 Drums stick appearance, 283
 Dry heat sterilization, 65–67
 Durham's tube 46f, 56
 Dwarf tapeworm, 639–640
E
Echinococcus granulosus, 636–638

ECHO viruses, 479
 Eclipse phase, 429
 Edward Jenner, 9
Ehrlichia, 405
 Eijkman test, 718
 Elek's gel precipitation test, 269–270
 Elementary body, 407
 Elephantiasis, 662
 El Tor *Vibrio*, 336
 Elution, 482
 Embryonated hen's egg, 431
 Encephalitis, 47
 Endemic haematuria, 642
 Endemic syphilis, 389
 Endemic typhus, 403
 Endemic, 109
 Endocytosis, 428
 Endospores, 27–28
 Endotoxin, 107
 Energy parasites, 407
 Enriched media, 43
 Enrichment media, 44
Entamoeba coli, 609t
Entamoeba histolytica, 598
 Enteric fever, 317–318
 Enterogaagregative *E. coli* (EAEC), 300–301
 Enterobacter, 303–304
 Enterobacteriaceae, 312–323
Enterobius vermicularis, 656–659
 Enterobiasis, 658
 Enterohaemorrhagic *E. coli* (EHEC), 301
 verotoxigenic *E. coli* (VTWC), 301
 Entero test, 612
 Enteroinvasive *E. coli* (EIEC), 301
 Enteropathogenic *E. coli* (EPEC), 300–301
 Enterotoxigenic *E. coli* (ETEC), 301
 Enterotoxin, 299
 Enteroviruses, 475
 Envelope, 422–423
 Enzyme-linked immunosorbent assay, 157–158
 indirect, 158
 sandwich, 156
 Epidemic, 109

Epidemic parotitis, 489
 Epidemic typhus, 403
 Epidermophyton, 555
 Episomes, 87
 Epithelial barriers, 124
 mucous membrane, 125
 skin, 124
 Epitope, 132
 Epstein–Barr virus, 469
 Erysipelothrix rhusiopathiae, 417
 Erythema nodosum leprosum, 381
 Erythrocytic schizogony, 621f
Escherichia coli, 297–311
 Espundia, 618
 Eukaryotes, 4
 Eumycetoma, 562
 Exoerythrocytic schizogony, 622
 Exotoxin, 107
 Explant culture, 432
 Exponential phase, 32
 Extrachromosomal genetic material, 87

F

Fab, 135
Fasciola hepatica, 600
 Fascioliasis, 81
 Favic chandelier, 542
 Fc, 135
 Fermentation, 34–35
 Fernandez reaction, 382
 Fever of unknown origin, 673
 Filariasis, 662
 Filtration, 72–73
 Fimbriae, 27
 Firm agar, 42
 Fir tree appearance, 284
 Fixed virus, 503t
 Flagella, 25
 Flagellates, 610–618
 Flaviviridae, 496
 Flavobacterium meningosepticum, 417
 Flea, 342
 Flocculation, 143–144
 slide flocculation, 145
 tube flocculation, 145
 Flotation techniques, 640

Fluctuation test, 90–91
Fonsecaea pedrosoi, 567
 Food poisoning, 280, 692
 Forssman antigen, 134
Francisella tularensis, 346
 Free-living amoebae, 606–609
 Fried egg appearance, 413
 Fungi imperfecti, 545
Fusarium spp., 564
Fusobacterium spp., 291

G

Gametocytes, 622
 Gametogony, 628
 Gamma-haemolysis, 245
Gardnerella vaginalis, 417
 Gas gangrene, 281
 Gas pak, 52
 Gene, 86
 General properties of viruses, 419–437
 Generation time, 31
 Genetic engineering, 97–98
 Genital warts, 531
 Genotype, 86, 89
 Genotypic variations, 89
 German measles, 532
 Germ tube test, 571
Giardia lamblia, 610
 Giardiasis, 612
 Glandular fever, 469
 Gonorrhoea, 262–263
 Graft versus host (GVH) reaction, 219
 Gram stain, 15–17
 Grave's diseases, 212
 Group B streptococci, 250–251
 Group D streptococci, 251
 Guarnieri bodies, 457
 Gummata, 386
 Guinea worm disease, 665–666
 Guinea worm, 665–666

H

HACEK group, 416–417
 Haemadsorption, 434
 Haemagglutination inhibition test, 426

- Haemagglutination test, 151
 Haemagglutination, 482–483
 Haemagglutinins, 425
 Haemocytolytic autoimmune diseases, 211–212
 Haemodigestion, 335
 Haemoflagellates, 614
 Haemolysis, 245
 Haemolytic anaemias, 211
Haemophilus spp., 357–362
 H. aegyptius, 357
 H. ducreyi, 362
 H. influenzae, 357
 Haemorrhagic fever, 535
 Hair perforation test, 557
 Halophilic vibrios, 339
 Hanging drop preparation, 15
 Hansen's disease, 378
 Hantaan virus, 535
 Hartmannella spp., 608
 Hashimoto's disease, 212
 HBcAg, 513
 HBeAg, 513
 HBsAg, 512
 Heavy chain disease, 141
 Heavy chains, 135–136
 Helical symmetry, 424
Helicobacter pylori, 418
 Helminths, 633–640
 nematelminths, 599
 platyhelminths, 599
 Hepatic amoebiasis, 605
 Hepatitis A virus (HAV), 509
 Hepatitis B virus (HBV), 511–512
 Hepatitis C virus (HCV), 439
 Hepatitis D virus (HDV), 517–518
 Hepatitis E virus (HEV), 518–519
 Hepatitis G virus (HGV), 519
 Hepatitis viruses, 509–519
 Herd immunity, 130, 222
 Herpes simplex virus, 461
 Herpes viruses, 460–471
 Heterogenic or heterophile specificity, 134
 Heterophile agglutination, 310
 Heterophile antibodies, 468
 Heterophile antigens, 134
 Heterotrophs, 30
 Hfr cells, 95
 Hib vaccine, 362
 Hidden or sequestered antigen theory, 210
 Hide porters disease, 274
 Histamine, 203
 Histocompatibility antigens, 177–178
Histoplasma capsulatum, 574
 Histoplasmosis, 574
 HLA complex, 177
 HLA typing, 178–179
 Hookworm disease, 653
 Hookworms, 652
Hortaea werneckii, 552
 Hospital acquired infections, 704
 Hospital infections, 704
 Hospital waste management, 79–83
 Host, 597
 Hot air sterilizer, 66
 Human immunodeficiency virus, 523–530
 Human leucocyte antigens, 176
 Human T cell lymphotropic virus-III, 523
 Humoral factors, 125
 Humoral immunity, 182
 Humoral immunodeficiencies, 196
 disorders of 196
 Hybridoma, 182
 Hybridoma technique, 135–137
 Hydatid cyst, 636
 Hydatid disease, 637
 Hydroclaving, 82–83
 Hydrogen sulphide production test, 59
 Hydrophobia, 504
 Hymenolepiasis, 640
Hymenolepis nana, 639–640
 Hypersensitivity, 200–209
 classification of, 200
 delayed, 200
 immediate, 200
 Hypnozoites, 619–620
- I**
- Iatrogenic infections, 706
 Iatrogenic transfer 106
 Icosahedral symmetry, 423–424
 ID-50, 109
 Id reaction, 557
 Idiosyncrasies, 205
 IgA, 139
 IgG, 138–139
 IgM, 140
 Immune complex mediated hypersensitivity, 206–207
 Immune cytolysis, 161
 Immune response, 180–193
 Immune response in malignancy, 128–129
 Immunity, 121–130
 Immunization, 222–226
 Immunizing agents, 222–226
 Immunoblotting, 158–159
 Immunodeficiency diseases, 194–199
 primary, 194–198
 secondary, 194
 Immunodiffusion test, 145
 Immunoelectron microscopy, 159
 Immunoelectrophoresis, 147
 Immunoenzyme test, 159
 Immunoferritin test, 159
 Immunofluorescence tests, 155
 direct, 155
 indirect, 156
 Immunogen, 131
 Immunoglobulin A, 139
 joining chain, 140
 secretory IgA, 139
 secretory piece, 139
 Immunoglobulin D, 140–141
 Immunoglobulin domains, 137
 Immunoglobulin E, 141
 Immunoglobulin G, 138–139
 Immunoglobulin M, 140
 Immunoglobulins, 135–141
 Immunological enhancement, 218–219
 Immunological surveillance, 220–221
 Immunological tolerance, 192
 mechanism of, 192–193
 Immunosuppressive agents, 185, 218
 cyclosporine, 218

Immunotherapy, 224–225
 Immunotherapy of cancer, 221
 IMViC tests, 56–61
 Incinerator, 81
 Inclusion bodies, 438
 Inclusion conjunctivitis, 409
 Inclusion granules, 23
 India ink, 14
 Individual immunity, 124
 Indole test, 56–57
 Infection, 102–109
 sources of, 103–105
 transmission of, 104
 types of, 102–103
 Infectious hepatitis, 510–511
 Infectious mononucleosis, 469
 Infectious waste, 79
 Inflammation, 126
 Influenza virus, 481–482
 Inhalation, 105
 Innate immunity 123–124
 Inoculation loop, 47
 Inspissation 67
 Interferon- α 190
 Interferon- β 191
 Interferon- γ 190
 Interferons, 442
 Interleukin-1, 188
 Interleukin-2, 189
 Interleukins, 188
 Intermediate host, 620
 Intestinal amoebiasis, 605
 Intestinal bilharziasis, 644
 Intestinal flagellates, 610–618
 Intestinal nematodes, 649–659
 Intestinal taeniasis, 633–634t
 In-use test, 78
 Iodine preparation, 606
 Iododeoxyuridine, 446
 Isoantigens, 133
 Isograft, 215
 Isospecificity, 133
Isospora belli, 631–632
 Ivanovasky, 8

J

Japanese encephalitis virus, 499
 Joseph Lister, 7
 Jumping genes, 87, 96

K

Kahn test, 145
 Kala azar, 631–632
 Kanagawa phenomenon, 340
 Kass concept/criteria, 698
 Kaufmann–White scheme, 316
 Killed vaccines, 223, 444–445
 Killer cells, 174
 Kirby–Bauer disc diffusion method, 116–117
Klebsiella, 304
 K. aerogens, 304
 K. oxytoca, 304
 K. ozaenae, 304
 K. pneumoniae, 304
 K. rhinoscleromatis, 304
 Koch's postulates, 7–8
 Koch sterilizer, 68
 Koplik's spots, 493
 Kuru, 537
 Kyasanur forest disease virus, 499–500

L

Laboratory diagnosis of
 bacterial diseases, 227–235
 Laboratory diagnosis
 of fungal diseases, 546–551
 Laboratory diagnosis of viral
 diseases, 447–450
Lac operon, 88–89
 Lactose fermenters, 297t
 Lag phase, 32
 Lancefield groups, 244f
 Larva migrans, 653
 Laryngoepiglottitis, 360
 Lassa fever virus, 535
 Late lactose fermenters, 297t
 Latex agglutination test, 151
 Lattice hypothesis, 143–144
 L-forms, 30
 Lawn culture, 48
 LD-50, 109
 LD body, 614–615
Leishmania brasiliensis, 617–618
Leishmania donovani, 614–617
 Leishmaniasis, 615

Leishmania tropica, 617
 Lepra cells, 378
 Lepra reaction, 318
 Lepromatous leprosy, 388
 Lepromin test, 382
Leptospira spp., 393f
 Leptospirosis, 394
 Life cycle of phages, 452
 Light chains, 135–136
 Lipid A, 21f
 Lipopolysaccharide, 21
 Listeria monocytogenes, 662
 Litmus milk test, 282
 Live vaccines, 222–223, 444
 Lobar pneumonia, 686
 Local anaphylaxis, 204–205
 Local immunity, 129–130
 Localized autoimmune
 diseases, 212–213
 Loeffler's serum slope, 266
 Loeffler's syndrome, 650
 Log phase, 32
 Louis Pasteur, 7
 Lowenstein–Jensen
 medium, 364
 Lower respiratory tract
 infections, 547
 Lyme's disease, 391–392
 Lymph nodes, 168–169
 Lymphadenopathy associated
 virus, 523
 Lymphatic filariasis, 662
 Lymphocyte-mediated
 reaction, 216
 Lymphocytes, 170
 Lymphogranuloma venereum, 409
 Lymphoid organs, 166
 Lymphokines activated killer
 cells, 174
 Lymphokines, 188
 Lysogenic conversion, 93, 454
 Lysogenic cycle, 454
 Lysogeny, 454
 Lysozyme, 125
 Lytic cycle, 452–453

M

Macrophages, 174–175
 histiocytes, 174
 monocytes, 174

- Madura foot, 561
 Maduramycosis, 561
Madurella mycetomatis, 561t
 Major histocompatibility complex, 176
 Malaria, 619–625
Malassezia furfur, 552
 Manson's blood fluke, 643
 Mantoux test, 371–372
 Marburg viruses, 535
 Mast cells, 202
 Mastigophora, 599
 Mc Intosh and Fildes's jar, 50
 Measles virus, 491
 Meat extract, 41
 Mechanism of graft rejection, 217f
 Mechanism of precipitation, 143–144
 Medical protozoology, 626–632
 Mediterranean fever, 403t
 Medusa head appearance, 273
 Membrane filter, 718
 Meningitis, 678–681
 aseptic, 678
 lymphocytic, 678f
 polymorphonuclear, 678f
 pyogenic meningitis, 678
 Meningococcaemia, 259
 Mesophiles, 31
 Mesosomes, 23
 Metachromatic granules, 265
 Metchnikoff, 10
 Methylene blue reduction test, 720
 Methyl red test, 57
 M–Fadyean's reaction, 272
 MHC restriction, 179
 Mickey mouse cap, 581
 Microbial pathogenicity, 106–107
 Microfilaria, 660f
 Microphages, 175
 basophils, 19
 eosinophils, 175
 granulocytes, 405
 neutrophils, 175
 polymorphonuclear leucocytes, 175
 Microscope, 11
 electron, 13
 Microscope (Continued)
 optical, 7
 bright field, 7–8
 compound, 7–8
 dark field, 9
 fluorescent, 13
 phase contrast, 8
 Microsporum, 555
 Microwaving, 82
 Migration-inhibiting factor, 190
 Migration inhibiting factor test, 191
 Milk borne diseases, 719
 Milk ring test, 417, 721
 Milker's nodes, 456t
 Minimum bactericidal concentration, 118
 Minimum infecting dose, 109
 Minimum inhibitory concentration, 118
 Minimum lethal dose, 109
 Miscellaneous bacteria, 416–418
 Miscellaneous viruses, 531–538
 MMR, 490
 Moist heat sterilization, 67–68
 Molar teeth appearance, 396
 Molluscum bodies, 459
 Molluscum contagiosum, 459
 Monkeypox, 458–459
 Monoclonal antibodies, 182–183
 Monokines, 188
 Monolayers, 432
 Morphological index, 378
 Most probable number (MPN), 34
 Moulds, 544t
 Mucor spp., 585
 Mucosa associated lymphoid tissue, 170
 Mumps, 489
 Mumps virus, 489
 Mutagenes, 90
 Mutational drug resistance, 95–96
 Mutations, 89–90
 frame shift, 89
 induced, 90
 Mutations (Continued)
 missense mutation, 90
 multisite, 90
 nonsense mutation, 90
 point mutation, 90
 spontaneous mutations, 89–90
 suppression mutation, 90
 Mycetism, 593
 Mycetismus, 593
 Mycetoma, 398, 561
 Mycobacteria, 363–373
 Mycobacterium avium, 378
 Mycobacterium bovis, 372
 Mycobacterium intracellulare, 378
 Mycobacterium leprae, 378–383
 Mycobacteria other than tubercle bacilli, 374
 Mycobacterium tuberculosis, 363–373
 Mycology, 539–551
 Mycoplasma, 412–415
 M. hominis, 412
 M. pneumoniae, 412
 Mycotoxicosis, 593
 Mycotoxins, 593
 N
 N. fowleri, 606
 Naegleria spp., 606–607
 NAG *Vibrio*, 337f
 Nagler reaction, 282–283
 Natural immunity, 123–124
 Natural killer cells, 174
 Nature of complement, 160
 Necator americanus, 655f
 Negative stain, 14, 252, 573
 Negri bodies, 438, 506
 Neil–Mooser reaction, 402
 Neisseria, 236–243
 N. gonorrhoeae, 260–261
 N. meningitidis, 257
 Neisser's stain, 265
 Nematodes, 599, 649–659
 Neural vaccines, 506
 beta propiolactone (BPL), 506
 Semple, 506

Neuraminidase, 426, 483
 Neutralization test, 154–155
 toxin neutralization, 155
 virus neutralization, 154
 NGU, 702
 Niacin test, 365–366
 Nichol's strain, 385
 NIH swab, 658f
 Nine-banded armadillo,
 379–380
 NNN medium, 616
Nocardia spp., 398
 N. asteroides, 397
 N. brasiliensis, 397
 N. caviae, 397
 Nocardiosis, 398
 Nonchromogens, 374
 Non-clostridial anaerobes,
 291
 Nongonococcal urethritis,
 702
 Noninfectious waste, 79
 Nonneural vaccines, 507
 human dipliod cell
 culture, 507
 Nonsense codons, 86
 Nonspecific defense, 217f
 Nonsporing anaerobes,
 291–296
 Normal microbial flora,
 110–114
 of conjunctiva, 112
 of gastrointestinal tract,
 113–114
 of genitourinary tract,
 114
 of mouth, 112
 of respiratory tract, 113
 of skin, 111
 Northern blotting, 101
 Norwalk virus, 534
 Nosocomial infections, 704
 Nucleocapsid, 422
 Nucleus, 23–24
 Null cells, 173–174
 Nutrition, 29

O

Occult filariasis, 662
 Oncogenes, 520–521
 Oncogenic viruses, 520–522
 Oocyst, 623f, 626f

Opportunistic infection in
 AIDS, 527
 Opportunistic mycoses,
 583–590
 Opsonization, 165
 Ophthalmia neonatorum, 410t
 Optochin sensitivity, 256t
 Oral polio vaccine (OPV),
 477–478
 Oral rehydration solution,
 692
 Oral thrush, 569
 ORE, 458
 Organ culture, 432
 Organ-specific antigens, 134
 Organ-specific autoimmune
 diseases, 212
 Organ specificity, 134
 Oriental sore, 617
Orientia, 400t
 Oroya fever, 406
 Orthomyxoviruses, 481–487
 Otomycosis, 592
 Ovens, 66
 Oxidase test, 60–61
 Oxidation-reduction
 potential, 35
 Oxidation, 34–35
 Oxidative phosphorylation, 34

P

Pandemic, 109
 Papillomavirus, 531
 Papova viruses, 531
Paracoccidioides brasiliensis,
 578
 Paracoccidioidomycosis,
 581–582
 Parainfluenza virus, 683
 Paramyxoviruses, 488–495
 Parasite, 597
 Parasites, 102
 Parasitology, 595–601
 Paratope, 132
 Paratyphoid fever, 317
 Park-Williams 267
 Parvovirus, 531
 Passive agglutination tests,
 151
 Passive immunity, 128–129
 artificially acquired, 128
 naturally acquired, 128

Passive immunization,
 224–225
Pasteurella, 346
 Pasteurization, 67
 Pathogenicity, 106–107
 Pathogens, 102
 Paul-Bunnell test, 470
 Paul Ehrlich, 8
 Peptidoglycan, 21
Peptococcus spp., 291
 Peptones, 41
Peptostreptococcus spp., 40
 Peripheral lymphoid organs,
 182
 Pernicious malaria, 631
 Petroff's method, 369
 Phaeohyphomycosis,
 567–568
 Phage conversion, 379
 Phagocytic cells, 174
 Phagocytosis, 125, 175
 Pharmacological mediators,
 202
 primary, 203–204
 secondary, 203–204
 Phenotype, 86
 Phenotypic variations,
 88–89
Phialophora verrucosa, 567
 Photochromogenes, 374
 Phototrophs, 30
 Picorna viruses, 475–480
 Piedra, 554
 black piedra, 554
 white piedra, 554
Piedraia hortae, 554
 Pili, 27
 Pinta, 390
 Pinworm, 656–659
 Pityriasis (Tinea) versicolor,
 552
 Plague, 343
 Plasma cells, 173
 Plasma technology, 83
 Plasmids, 87
Plasmodium spp., 599
 P. falciparum, 619
 P. malariae, 619
 P. ovale, 619
 P. vivax, 619
 Pneumococci, 252
Pneumocystis carinii, 587

- Pneumocystosis, 587–588
 Pneumonia, 685
 Pneumonic plague, 344
 Poliomyelitis, 477
 Poliovirus, 481–482
 Polymerase chain reaction, 100–101
 Ponder's stain, 265
 Pork tapeworm, 633–636
Porphyromonas spp., 291
 Post kala azar dermal leishmaniasis, 624
 Postzone, 143
 Pour plate culture, 49
 Pour plate method, 698
 Poxviruses, 456–459
 Precipitation reactions, 143–144
 Premunition, 127
Prevotella spp., 291
 Primary amoebic meningoencephalitis, 607
 Primary atypical pneumonia, 414
 Primary cell cultures, 433
 Primary immune response, 180–181
 Prions, 437, 536
 Proglottides, 599
 Prokaryotes, 4
 Prophage, 454
 Prosodemic, 109
 Proteaceae, 307
 Protein A, 239
Proteus, 307
 P. mirabilis, 307
 P. vulgaris, 307
 Protista, 21, 36
 Protoplasts, 22
 Protozoa, 626–632
Providencia, 311
 Prozone, 143
 Pseudocyst, 627
 Pseudohyphae, 541
Pseudomonas, 329–333
 Pseudophyllidean cestodes, 600
 Psittacosis, 412
 Psychrophiles, 31
 Pulmonary tuberculosis, 365f
 PUO, 673–677
 Purine bases, 84
 Pyaemia, 109, 669
 Pyocyanin, 329
 Pyogenic bacteria, 669
 Pyogenic infections, 310
 Pyrexia of unknown origin, 673
 Pyrimidine bases, 84
 Pyrogens, 674

Q
 Q fever, 404
 Quellung reaction, 252

R
 Rabies virus, 502
 Rabies, 37
 Racial immunity, 124
 Radial immunodiffusion, 146
 Radiation, 71–72
 ionizing, 71
 nonionizing, 71
 Radioimmunoassay, 156–157
 Ranikhet virus, 491
 Rapid growers, 374
 Reaginic antibodies, 202
 Recombinant vaccines, 224, 445
 Reiter's strain, 388
 Relapsing fever, 390
 Replica plating method, 91–92
 Replication of viruses, 427–428
 Respiratory syncytial virus, 494
 Respiratory tract infections, 682–688
 Retrovirus, 523
 Reverse passive agglutination, 151
 Reverse transcriptase enzyme, 523
 Reynold–Braude phenomenon, 571
 Rhabdoviruses, 502–508
 Rheumatoid arthritis, 213
 Rheumatoid factor, 213
 Rhinoscleroma, 304
 Rhinosporidiosis, 554
Rhinosporidium seeberi, 564
 Rhinovirus, 480
 Rhizopoda, 598
Rhizopus spp., 585
 Ribosomes, 23
 Rickettsia, 400–406
 Rickettsiaceae, 400–406
 Rideal–Walker test, 78
 RNA PCR, 100–101
 RNA viruses, 436
 Robert Koch, 7–8
 Robertson's cooked meat medium, 53
 Rocket electrophoresis, 148
 Rocky mountain spotted fever, 403
 Rotavirus, 533
 Round worm, 104
 RTF 95, 96
 Rubella virus, 531
 Rubella, 531

S
 Sabin and Feldman dye test, 628
 Safety-pin appearance, 341
 Saline preparation, 606
 Salmonella gastroenteritis, 318
 Salmonella septicaemia, 318
Salmonella, 313
 S. cholerae-suis, 312
 S. paratyphi A, 313
 S. paratyphi B, 312
 S. paratyphi C, 312
 S. typhi, 312
 S. typhimurium, 312
 Sand flies, 615
 Saprophytes, 102
 SARS, 534
 Satellitism, 358–359
Schistosoma haematobium, 600
Schistosoma japonicum, 600
Schistosoma mansoni, 600
 Schistosomal dysentery, 644
 Schistosomiasis haematobia, 642
 Schistosomiasis japonica, 645
 Schistosomiasis mansoni, 644

- Schultz-Dale phenomenon, 205
- Scotochromogens, 374
- Scrub typhus, 402
- Seat worm, 656-659
- Secondary immune response, 181-182
- Sedimentation techniques, 5
- Sedormid purpura, 206, 212
- Segregation, 79-80
- Seitz filter, 73
- Selective media, 44
- Semi-defined media, 43
- Semi-solid medium, 42
- Sensitivity, 143
- Septicaemia 109, 667-672
- Septicaemic plague, 343
- Sereny test, 301
- Serological reactions, 142
- Serratia, 303-304
- Serum hepatitis, 514
- Serum sickness, 207-208
- Settle plate method, 722
- Sex pili, 27
- Sexually transmitted diseases, 700
- Shared or cross reacting antigens, 211
- Shigella*, 324-328
Sh. boydii, 326
Sh. dysenteriae, 325
Sh. flexneri, 324
Sh. sonnei, 326
- Shigellosis, 326
- Shwartzman reaction, 209, 331
- Significant bacteriuria, 695
- Simple carriers, 514
- Simple media, 42
- Sintered glass filters, 73
- Skin reactive factor, 191
- Slide agglutination test, 149
- Slide culture, 550
- Slime layer, 24
- Slit sampler method, 722
- Slow reactive substance, 203
- Slow virus diseases, 535
- Slow viruses, 536
- Small poxvirus, 457
- Sore throat, 248, 684
- Southern blotting, 101
- Space vehicle appearance, 472
- Species immunity, 123-124
- Species specificity, 133
- Specificity, 143
- Spheroplasts, 22
- Spherule, 579
- Spirillum minus*, 418
- Spirochaetes, 384-395
- Spleen, 169-170
- Split tolerance, 193
- Sporadic, 97
- Sporothrix schenckii*, 565
- Sporotrichosis, 565
- Sporozoa, 599
- Sporozoites, 589
- Spotted fever, 402
- Stab culture, 49
- Staph. epidermidis*, 243
- Staph. saprophyticus*, 243
- Staph. aureus*, 236
- Staphylococcal scalded skin syndrome, 241
- Stationary phase, 32
- STD, 700
- Stem cell deficiency, 14-15
- Stenotrophomonas maltophilia*, 332-333
- Sterilization, 64-78
 physical methods, 64
- Stimulatory hypersensitivity, 209
- Stokes disc diffusion method, 117
- Streak culture, 48
- Street virus, 38
- Streptobacillus moniliformis*, 418
- Streptococcal MG test, 240
- Streptococci, 236
- Streptococcus agalactiae*, 250
- Streptococcus pyogenes*, 236
- Streptococcus viridans*, 251
- Streptodornase, 247
- Streptokinase, 247
- Streptolysin O, 246
- Streptolysin S, 247
- Streptomyces, 398-399
- String of pearls reaction, 273
- String test, 336
- Stroke culture, 49
- Structure and function of immune system, 166-179
- Structure of DNA, 84
- Structure of immunoglobulins, 135
- Subacute sclerosing panencephalitis, 537
- Subcutaneous mycoses, 561
- Subunit vaccines, 224, 369
- Sugar fermentation reactions, 56
- Sugar media, 46
- Sun ray appearance, 330
- Super carriers, 514
- Superficial mycoses, 552-560
- Supravital staining, 14
 impregnation, 14
 negative, 14
 simple, 14
- Swarming, 308
- Sweep plate, 723
- Swine flu, 485
- Syphilis, 385-386
- Systemic anaphylaxis, 204
- Systemic autoimmune diseases, 213-214
- Systemic lupus erythematosus (SLE), 213
- T**
- T-dependent Ags, 180-182
- T-independent Ags, 182
- Tzanck cells, 464
- TAB vaccine, 323
- Taenia echinococcus*, 65
- Taenia saginata*, 633-636
- Taenia solium*, 633-636
- Tanapox, 459
- Taxonomy, 36
- T-cells, 180
- Temperate phage, 454
- Tetanolysin, 284
- Tetanospasmin, 284
- Tetanus, 285
- T-helper cells, 172
 instructive theories, 186
 direct template, 186
 indirect template, 186
 selective theories, 186-187
 clonal selection, 187
 natural selection, 186-187
 side chain theory, 186

- Thermophiles, 53
 Threadworm, 656–659
 Thrombocytopenic purpura, 206, 212
 Thymus, 168
 Thyrotoxicosis, 212
 Tinea barbae, 556
 Tinea capitis, 556
 favus, 556
 Tinea corporis, 556
 Tinea cruris, 556
 Tinea imbricata, 556
 Tinea manuum, 556
 Tinea nigra, 553–554
 Tinea pedis, 556
 Tinea unguium, 557
 Tinea versicolor, 552–553
 Tissue culture, 432
 Tissue cyst, 626
 Tissue nematodes, 660–666
 Titre, 142
 T-lymphocytes, 172
 Toxaemia, 660–661
 Toxic shock syndrome, 241
 Toxigenicity, 107–108
 Toxoid, 128, 223
Toxoplasma gondii, 626–628
 Toxoplasmosis, 627
 Trachoma inclusion conjunctivitis (TRIC) agents, 407
 Trachoma, 409
 Transcription, 86
 Transduction, 92–93
 Transfection, 521
 Transfer factor, 190
 Transferable (infectious) drug resistance, 96
 Transformation, 92–93, 254
 Translation, 86
 Transplantation immunity, 215
 Transplants, 215–216
 types of, 215–216
 Transport media, 46
 Transposition, 87, 96
 Transposons, 87, 96
 Traveller's diarrhoea, 301, 689
 Treatment of waste, 80
 Trematodes, 641–648
 Trench fever, 406
Treponema pallidum agglutination (TPA) test, 389
Treponema pallidum haemagglutination (TPHA) test, 389
Treponema pallidum immune adherence (TPIA) test, 389
Treponema pallidum, 412
Treponema pertenue, 390
Trichomonas vaginalis, 613f
 Trichomoniasis, 613
 Trichophyton, 555
Trichosporon beigeli, 554
 Triphenyl tetrazolium chloride test, 697
 Triple sugar iron, 62–63
 T-suppressor cells, 172
 Tube dilution method, 233
 Tuberculin test, 371–372
 Tuberculoid leprosy, 381t
 Tuberculosis, 367
 Tularemia, 346
 Tumour antigens, 219–220
 Tumour immunity, 219–220
 Tumour necrosis factor – γ , 191
 Tween 80 hydrolysis, 367
 Two dimensional immunoelectrophoresis, 149f
 Tyndallization, 68
 Type I hypersensitivity, 201–202
 Type II hypersensitivity, 205–206
 Type III hypersensitivity, 206–208
 Type IV hypersensitivity, 208–209
 contact dermatitis type, 209
 tuberculin (infection) type, 208
 Type V hypersensitivity, 209
 Typhoid fever, 317
 Typhoral, 323
 Typhus fever, 403
- U**
 Ultraviolet radiations, 71
 Undulant fever, 353
 Upper respiratory tract infections, 683
- Ureaplasma urealyticum*, 418
 Urease test, 75–76
 Urinary schistosomiasis, 642
 Urinary tract infections, 300, 302–303, 695–699
- V**
 Vaccine bath, 67
 Vaccines 222, 444
 Vaccinia, 456t
 Varicella-zoster virus, 465
 Vectors, 104
Veillonella spp., 291
 Venereal disease research laboratory test, (VDRL), 387–388
 Verocytotoxin, 326
 Vertical transmission, 293
 V-factor, 358
 Vi antigen, 314–315
Vibrio cholerae, 334
 Vincent's angina, 392–393
 Viral assays, 435–436
 Viral genetics, 436
 Viral haemagglutination test, 426–427
 Virion, 421
 Viroids, 437
 Virology, 421
 Viropexis, 428
 Virulence factors, 120
 Virulent cycle, 452
 Viruses, 419–437
 Virus–host interaction, 438–446
 Visna, 536
 Vital staining, 14
 Voges-Proskauer test, 57–58
 Volutin granules, 265
 Von Mognus phenomenon, 429
- W**
 Waksman, 8
 Waldenstrom's macroglobulinaemia, 141
 Wasserman test, 388
 Water bath, 67
 Waterhouse-Friederichsen syndrome, 258
 Weil-Felix test, 676

West nile virus, 501
West's post nasal swab, 349
Western blot, 560, 159
Wet mount, 14-15
Wheal and flare reaction,
204
Whipworm, 654-656
Whooping cough, 348
Widal test, 321-322
Wood's lamp, 553
Wool sorter's disease, 274
Wuchereria bancrofti,
660-663
Wuchereriosis, 661

X

X-factor, 357-358
Xenodiagnosis, 663
Xenograft (heterograft), 216
Xenopsylla cheopis, 342
X-linked agammaglobulinae-
mia (Bruton's disease),
196

Y

Yaws, 390
Yeast extract, 41
Yeast-like fungi, 545

Yeasts, 544

Yersinia, 341

Y. enterocolitica, 345-346
Y. pestis, 341
Y. pseudotuberculosis, 341

Z

Zidovudine, 446
Ziehl-Neelsen stain, 17-18
Zone phenomenon, 143
Zoonoses, 710
Zoonotic diseases, 712
Zygomycosis, 585-586